# APPLICATION OF METABOLOMICS IN ASSESSMENT OF FUNCTIONAL DIVERSITY AND QUALITY TRAITS OF SELECTED AFRICAN SOLANACEAE ACCESSIONS

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# Application of metabolomics in assessment of functional diversity and quality traits of selected African solanaceae accessions

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## DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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This thesis has been submitted for examination with our approval as university supervisors.

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## DEDICATION

I dedicate this thesis to my loving wife Mrs. Susan Jeptoo Mibei, daughters Faith Jepchumba and Joy Jepkemboi, to parents Mr. Henry Sitienei and Mrs. Esther Sitienei, brothers, sisters and friends for their encouragement, moral support, prayers and love. To my supervisors who have been an inspiration to me in carrying out this study. Finally to men and women who cherish the knowledge of science. GOD bless you ALL.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of variance |
| AVRDC | Asian Vegetable Research Development Centre |
| bwt | Body weight |
| CGA | Chlorogenic acid |
| COMESA | Common Market for Eastern and Southern Africa |
| CVD | Cardiovascular disease |
| EI | Electron ionization |
| FAO | Food and Agriculture Organization |
| GABA | Gamma-aminobutyric acid |
| GC-MS | Gas chromatography - mass spectrometer |
| GC-MS | Gas chromatography – Mass spectrophotometer |
| HCA | Hierarchical cluster analysis |
| HPLC | High performance liquid chromatography |
| JKUAT | Jomo Kenyatta University of Agriculture and Technology |
| KALRO | Kenya Agricultural and Livestock Research Organization |
| LC-MS | Liquid chromatography - mass spectrometer |
| MS | mass spectrometer |
| MSTFA | N-methyl-N-(trimethylsilyl) trifluoroacetamide |
| NMR | Nuclear magnetic resonance |
| PCA | Principal Component Analysis |
| PDA | Photo Array Detector |
| ROS | Reactive oxygen species |
| RSA | Radical scavenging activity |
| Rt | Retention time |
| RT | Room temperature |
| SAS | Statistical analysis software |
| SPSS | Statistical package for social scientists |
| TCA | Tricarboxylic acid |
| TOF | Time-of-flight detectors |
| USA | United States of America |
| UV-Vis | Ultraviolet - visible |
| WHO | World Health Organization |

## DEFINITION OF TERMS

**Abiotic stress –** the negative impact of non-living factors on the living organisms in a specific environment such as drought, extreme temperatures (cold, frost and heat), salinity, sun exposure, high wind—which can have an adverse effects on an ecosystem.

**Accession –** is a distinct, uniquely identifiable sample of seeds representing a cultivar, breeding line or a population, collected from a particular area and maintained in storage for conservation and use.

**Accession number –** is a unique identifier that is assigned by the custodian when an accession is entered into a gene bank and should never be assigned to another accession.

**Biotic stress -** is stress that occurs as a result of damage done to plants by other living organisms, (examples) such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds, and cultivated or native plants.

**Breaker stage –** is the stage where there is a definite "break" in colour from green to tannish yellow, pink or red on not more than 10% of the surface.

**Diabetes –** a disease in which the body's ability to produce or respond to the hormone insulin is impaired, resulting in abnormal metabolism of carbohydrates and elevated levels of glucose in the blood.

**Antidiabetic -** A substance that helps a person with diabetes control their level of glucose (sugar) in the blood. Antidiabetic agents include insulin and the oral hypoglycemic agents.

**Drought –** a prolonged period of dry weather or abnormally low rainfall, leading to a shortage of water.

**Metabolomics -** is the comprehensive, qualitative, and quantitative study of all the small molecules (the metabolome) in an organism

**Metabolome –** is the entire biochemical complement present within an organism

**Metabolite fingerprinting –** is rapid and high-throughput methods where global metabolite profiles are obtained from crude samples or simple cellular extracts. In general, metabolites are neither quantified nor identified

**Metabolite profiling -** is the identification and quantification of metabolites related through their metabolic pathway(s) or similarities in their chemistry

**Nutraceutical –** a food or part of a food that provides medical or health benefits including the prevention and/or treatment of disease in addition to its basic nutritional value

**Tolerance** – this is the ability of plants to mitigate the negative fitness effects caused by herbivory. It is one of the general plant defense strategies against herbivores, the other being resistance, which is the ability of plants to prevent damage

## LIST OF PUBLICATIONS AND PROCEEDINGS

1. **Elias K. Mibei,** Willis O. Owino, Jane A. mbuko, James J. Giovannoni and Arnold N. Onyango (2017). Metabolomic analyses to evaluate the effect of drought stress on selected African Eggplant accessions. *Journal of the Science of Food and Agriculture. (wileyonlinelibrary.com)DOI10.1002/jsfa.8458*

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## ABSTRACT

African eggplants and tomatoes are among the most economically important and valuable vegetable fruit crops which have played an important historical role in the traditional diets in Africa. Their nutritional quality and diversified metabolites which contribute to their usefulness as food and the effect of drought stress on these metabolites has not been well defined. The study aimed at application of metabolomics in identification and characterization of metabolites in African eggplant and tomatoes under drought stress and assessment of antidiabetic activities of fruits of five selected African eggplant accessions. Seeds of the two accessions were obtained from World Vegetable Centre (AVRDC), Arusha, Tanzania. They were grown in the greenhouse and subjected to drought stress. Fresh African eggplant leaves were sampled at three different growth stages, namely before stress, 2 weeks and 4 weeks after stress. African eggplant and tomato fruits were harvested at three stages of ripening; mature green, breaker and mature red stages. Ascorbic acid and carotenoid contents were analyzed using HPLC. Ascorbic acid was seen to increase considerably during growth and fruit ripening, and it was significantly high in mature stages (week 4) and mature red fruits ($p < 0.05$). The most pronounced difference was also observed between control and stressed leaves where high ascorbic acid levels was observed in drought stressed GBK50591 leaves (28.49±1.20mg/100g), RV100265 fruits (23.57±2.81 mg/100g) and V1035028 fruits (44.42±0.58 mg/100g). Major carotenoids such as Xanthophylls (neoxanthin, violaxanthin, zeaxanthin and lutein) and carotenes (β–carotene and α–carotene), phytofluene, lycopene, phytoene as well as chlorophylls (chlorophyll-b and Chlorophyll-a) increased with growth and ripening stage of the crops. Although the leaves of the stressed crops reported decreased amount of carotenes, chlorophylls, neoxanthin and violaxanthin, the concentration of zeaxanthin increased with stress whereas lutein had no significant change ($p > 0.05$). There was an increase in lycopene during fruit ripening and a decrease in chlorophylls (a and b). Beta carotene increased, neoxanthin slightly decreased, while lutein was virtually constant during development. The total carotenoid contents varied from 1.07±0.16–23.21±4.61 mg/100g and 1.89±0.88–108.14±1.90 mg/100g for the African eggplant and tomato fruit tissues, respectively. Metabolite analyses were carried using a GC–MS and LC–MS and metabolite identification carried out with the Golm, Germany metabolomics library software. Proline, glutamate, sucrose, fructose and tricarboxylic acid cycle metabolites were shown to significantly increase with stress ($p < 0.05$). Principal component analysis (PCA) showed a clear discrimination between the different accessions, growth stages, and stress/control conditions.

The results for antidiabetic studies showed that immediately after diabetes induction, the blood glucose was elevated in the mice and administration of the extracts significantly ($p < 0.05$) reduced the blood glucose levels. The effect was dose dependent with 300 mg/kg dosage showing good activity and maintaining the levels within the normal level. Metformin treated mice recorded the biggest fall in blood glucose levels (48.08 %) followed by RV10333 (44.86 %), RV10511 (41.66 %), RV10265 (40.01 %), RV101201 (35.23 %) and RV10445 (17.23 %). In addition, the treatment with the extracts did not induce any signs of toxicity. The results of this study illustrates the common aspects associated with drought stress effects on vegetable quality and indicate that drought stress affects the concentration of ascorbic acid and carotenoids as well as metabolites such as amino acids, sugars and organic acids. The findings also indicate that harvesting the leaves and fruits at mature stages has improved nutritional benefit. The study also suggests that the African eggplant fruits possess antidiabetic properties and their consumption may be important in management of diabetes.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background information

The Solanaceae comprises a number of economically important food crops. These crops are important to agriculture, food security, human nutrition and health (Ray-Yu and Ojiewo, 2011). They include globally-consumed peppers (*Capsicum* sp), potato (*Solanum tuberosum*), cultivated tomato (*S. lycopersicum*), wild tomato (*S. hirsutum, S. peruvianum and S. pennellii*), cultivated eggplant (*S. melongena*), African eggplants (*S. aethiopicum, S. macropcarpon* and *S. anguivi*) and African nightshades (*S. scabrum* and *S. villosum*) (Knapp *et al.*, 2004). Many members of the family contain potent alkaloids, and some are highly toxic, but many, including tomatoes, potatoes, eggplant, bell/chili peppers, and tobacco are widely consumed. Although their fruits and vegetables are widely used, the leaves of these plants can be toxic to humans due to the presence of alkaloids, such as $\alpha$-tomatine in tomato leaves. The rich source of alkaloids and other secondary metabolites makes Solanaceae plant species have a high potential for drug discovery (Lee, 2007).

Availability of optimum amount of water during whole plant life cycle is an essential element for its better growth and yield production (Nair *et al.*, 2008). It is understandable that limited water availability at any stage of plant growth and development can stimulate a number of plant processes including change in metabolism, physiology and morphology (Ashraf *et al.*, 2011) hence may be detrimental to food production. Drought is a serious threat to food production and is a global problem which affects 64% of global land area (Rabara et al., 2014). Its frequency and intensity are expected to increase due to looming threat of climate change (Kang *et al.*, 2009). The Solanaceae crops are well adapted to almost all climatic regions of the world. As an adaptive response to drought stress, crops triggers accumulation of important metabolites to tolerate stress. These metabolites are the link between genotype and phenotype (Fiehn, 2002) and are considered the ultimate phenotype of cells deduced by the perturbation of gene expression in response to the environment (Saito and Matsuda, 2010). Understanding how solanaceae respond to water deficit is therefore important in order to develop crops tolerant to drought. One approach

to elucidate plants response to water stress is through metabolomics; a powerful tool to gain insights into how plant metabolic processes are regulated under stressful growing conditions (Obata and Fernie, 2012).

### 1.2 Problem statement

For centuries, wild Solanaceae crops have comprised an important segment of food security crops among African communities (AVRDC, 2008; Ray-Yu and Ojiewo, 2011). They are important for nutritional contributions and also health promoting properties, and can therefore be used as functional foods (Gebhardt, 2016). However, their seasonality of production, loss of indigenous knowledge, a dearth of information on potential nutraceutical benefits and changing food habits has progressively denigrated their dietary and economic importance. As a result, the wild Solanaceae have been neglected for many years and if the situation is left unchecked it might result to underutilization and loss of its biodiversity. To better explore and exploit the nutritional and nutraceutical potential of wild Solanaceae, there is need for research to establish the metabolomic profiles and hypoglycemic potential of these species. The greater use of metabolomics could assist in the study of the active secondary metabolites from these crops as novel or improved phytotherapeutic agents in relation to their hypoglycemic activity. This can also contribute to the development of novel functional and health promoting foods.

### 1.3 Justification

Solanaceae crops are popularly consumed in almost all the states of the Common Market for Eastern and Southern Africa (COMESA). Numerous epidemiological studies have shown that a diet rich in vegetables and fruits has significant protective effects for curbing metabolic diseases such as diabetes and cardiovascular disorders. In spite of a large body of evidence confirming nutritional contributions, health properties and stress tolerance of solanaceae crops, there has been very little concerted effort towards exploiting this bio-diverse nutritional and health resource to address the complex food security and health problems of sub Saharan Africa. Thus, data on the isolation, identification and characterization of the various metabolites in these Solanaceae crops is limited. There is therefore a need to close this knowledge gap as increasing global attention is turned towards mobilizing local biodiversity for food security and health. This necessitates credible metabolite profiling of bioactive phytochemicals in these crops. On the

other hand, considerable research has been done in order to understand how plants respond to drought stress. One approach to elucidate plants response to water stress is through metabolomics.

This research involved the study of the metabolomic profiles of two African Solanaceae crops, particularly the tomato and eggplant. The envisaged activities involved elucidating the metabolites responsible for stress tolerance and targeting bioactive metabolites with hypoglycemic potential. The increased levels of bioactive metabolites in these crops will suggest that these crops could be exploited as a potential commercial product with enhanced nutritional value. The knowledge from the study will explore the precious assets of Solanaceae crops thus help to revive and promote their consumption within Kenya for food security, poverty reduction, and sustainable land use.

## 1.4 Objectives

### 1.4.1 General objective

To characterize the metabolites in selected African eggplant and tomato accessions under drought stress and study antidiabetic potential of selected African eggplant accessions.

### 1.4.2 Specific objectives

i) To analyze the ascorbic acid and carotenoid contents of the leaf and fruit tissues of the selected African Solanaceae accessions subjected to drought stress during specific stages of growth and ripening;

ii) To carry out qualitative and quantitative profiling of metabolites in leaf and fruit tissues of the selected African Solanaceae subjected to drought stress at specific stages of growth and ripening;

iii) To determine the antidiabetic activity of fruits of selected African eggplant accessions.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Solanaceae Crops

Solanaceae crops, particularly tomatoes and eggplants are important vegetable crops of widespread popularity. Because of their potential for large-scale production, they have excelled in the world as major food crops (Samuels, 2015). They are consumed as both leafy and fruit vegetables in diverse ways, including raw, as an ingredient in salads and sauces in many dishes (AVRDC, 2008; Denton and Nwangburuka, 2011). They have been reported to contain health building substances particularly essential minerals, vitamins and antioxidant compounds (Denton and Nwangburuka, 2011) therefore plays an important role in maintaining good health and reducing the risk of illness. The consumption of Solanaceae vegetable and fruit has been associated with decreased risk of degenerative disease such as diabetes and cardiovascular diseases (Zhang *et al.*, 2009). Therefore these crops are recommended as healthy and functional food for their nutritional and medicinal value (Yang and Keding, 2009).

A diverse of wild or indigenous vegetable species exists throughout Africa (Schippers, 2000). Several species of the Solanaceae family have been selected and developed as food plants. They are widely distributed and consumed in the tropical areas of sub-Saharan Africa, throughout the Middle East into Asia, Brazil and Southern Europe (Schippers, 2000). African Solanaceae, the wild relatives of the cultivated Solanaceae are among the nutritionally important and valuable crops consumed in Africa (Chadha and Mndiga, 2007). Most have been introduced and distributed throughout the Middle East into India, Asia and South America, primarily Brazil (Daunay *et al.*, 2001). They are cultivated for their edible fruits and leaves and are of great significance because of their high nutritional value.

Additionally, their fruits are increasingly becoming a significant component of the human diet worldwide and have been reported to be an excellent source of several nutrients and metabolites such as organic acids, sugars, amino acids and vitamins.

### 2.1.1 Tomato (*Solanum lycopersicum* L.)

Tomato (*Lycopersicon esculentum* L.) is one of the most important horticultural crops of great interest in the world (Gerszberg and Hnatuszko‐Konka, 2017). It originated in western South America (Blanca *et al.*, 2012), and domestication is thought to have occurred in Central America (Naika *et al.*, 2005). In 2013 tomato was 7$^{th}$ in global production, achieving a world production of approximately 164,000,000.00 million tonnes on a total area of nearly 4.8 million hectares (FAOSTAT, 2013). It is an annual herb with an erect to prostrate stems. It has a strong taproot with dense lateral and adventitious roots. The stem is solid, coarsely hairy and glandular. The leaves are arranged spirally, with no stipules while the petiole length varies from 3 to 6 cm (Van der Vossen *et al.*, 2004). The leaflets vary in size and are irregularly toothed. Flowers are bisexual and regular in shape and often with a yellow corolla (Van der Vossen *et al.*, 2004). Closed stigma and style enhances autogamy and reduces chances of crossing. The fruit is a berry usually red but may sometime vary from pink, orange to yellow when ripe (Van der Vossen *et al.*, 2004).

Tomato is among the most widely consumed vegetable crops grown worldwide for its edible fruits. Its fruits are consumed fresh or as processed products such as canned tomato, paste, sauce, juice ketchup, stews and soup (Ray *et al.*, 2011). Tomato fruits are of great significance because of their high nutritional value (Ezura, 2009). They are an excellent source of several nutrients, antioxidants primarily carotenoids such as lycopene, $\beta$-carotene (pro-vitamin A) and vitamin C and secondary metabolites that are important for human health: folate, flavonoids and phenols (Wilcox *et al.*, 2003). As the richest source of lycopene, consumption of tomatoes has been reported to protect cells from oxidants that have been linked to human cancer (Mutanen *et al.*, 2011). In addition tomato has chlorogenic acid which has been shown to have hepatoprotective, hypoglycemic and antiviral activities (Park *et al.*, 2010). Epidemiological studies have shown that consumption of raw tomato and its tomato based products is associated with a reduced risk of chronic degenerative diseases (Agarwa and Aai, 2000) such as cancer and cardiovascular diseases (Tamagnone *et al.*, 1998; Giovannucci *et al.*, 2002). This protective effect has been mainly attributed to its valuable bioactive components flavonoids and phenols (Tan *et al.*, 2010) with antioxidant properties (Borguini and Torres, 2009).

Being a tropical plant, tomato is well adapted to almost all climatic regions of the world; however, environmental stress factors are the primary constraints of this crop's yield potential (Gerszberg and Hnatuszko‐Konka, 2017). Its domestication for human consumption has led to an inevitable loss in genetic variance (Schauer *et al.*, 2005), and allows the domesticated species to be more susceptible to plant pathogens. It is also self-pollinating, but can easily hybridize within the species or cross with wild relatives under appropriate conditions, thus permitting gene introgression from wild relatives (Harold *et al.*, 2007). Recently, significant progress has been made to improve the levels of human health-promoting compounds in tomato fruits through metabolic engineering or breeding (Rosati *et al.*, 2000).

#### 2.1.1.1 Wild tomato species

Wild tomato plants are herbaceous species, mainly native to South America, particularly across the regions of Peru, Chile, Ecuador, Colombia and the Galapagos Islands (Kole, 2007). The main examples of the wild species are *Solanum hirsutum, S. peruvianum* and *S. pennellii*. They are edible and have been reported to be used by the indigenous people for medicinal purposes (Grandillo and Chetelat, 2011). In addition, they are a rich source of genes, which harbor genetic diversity that yields heritable variation in fruit chemistry. This could be exploited to identify genes regulating their synthesis and accumulation (Lee *et al.*, 2012). They have different phenotypes based on their different geographical distribution which is reflected in their genetic diversity. Physical barriers such as deserts and mountains have kept the wild tomato species genetically distinct (Grandillo and Chetelat, 2011). There are 13 recognized wild tomato species that display a great variety of phenotypes and can be crossed with the cultivated tomato (Peralta *et al.*, 2008). These wild tomatoes are important for breeding, as sources of desirable traits, and for evolutionary studies (Bolger *et al.*, 2014). The chemical composition of wild tomato crops have been reported to influence resistance to many tomato insect pests (Tohge and Fernie, 2015). Some studies have been done using the wild tomato species to genetically improve the domesticated tomato which has a poor genetic diversity from inbreeding during the domestication of the tomato (Zhang *et al.*, 2006). This has led to investigations into the secondary metabolites produced such as phenolic properties of the wild tomato relatives (Antonious *et al.*, 2003).

### 2.1.2 Eggplant (*Solanum melongena* L.)

The eggplant (*Solanum melongena* L.) is a short-lived perennial herb that belongs to the family Solanaceae (Grubben and Denton, 2004). It is one of the most widespread vegetables in the world originating from Eastern countries and Asia (Denton and Nwangburuka, 2011). It is domesticated and grown as an annual plant and is one of the most consumed fruit vegetables in tropical Africa; probably the third after tomato and onion, and before okra (Grubben and Denton, 2004).

The eggplant is cultivated as an annual plant in temperate climate but it is actually a tropical perennial crop. Its growth varies with accession and environment with a minimum height of 40 cm and the tallest accession being 150 cm tall, most of the leaves were large and coarsely lobed with leaf breadth ranging from 5 to 10 cm while leaf length varied from 10 to 20 cm (Kouassi *et al.*, 2014). The eggplants that are not domesticated have large leaves over 15 cm broad and 30 cm long with a plant height of up to 225 cm. They have purple to white flowers with 5 lobed corollas, also called 5-merous (5 stamens, 5 sepals, 5 petals) and they have spiny stems. They mostly have yellow stamens but the round-fruited and globose cultivars have 6, 7 or 8 merous flowers. Kalloo (1993) describes the eggplant as a self-pollinated crop, but sometimes cross-pollination occurs and parthenocarpy sometimes occur (Boyaci *et al.*, 2011). Their fruits can be as long as 30 cm which is exceptionally large compared to other wild types which can be less than 3 cm in breadth (Swarup, 1995; Hurtado *et al.*, 2012). There is a big diversity of eggplant cultivars on the market varying in shape and colour, the most common ones being dark purple or violet

#### 2.1.2.1 African eggplant species

African eggplants are rare ethnomedicinal herbs and the wild relatives of the cultivated eggplants (Sękara *et al.*, 2007; Stàgel *et al.*, 2008). They constitute important fruit and leaf vegetables in Africa (Schippers, 2000) due to their dual value with leaves and fruits being edible. While the brinjal eggplant (*Solanum melongena*) originated in India (Tindall, 2008), the African eggplants are probably native to Africa (Bukenya and Carasco, 1999). They are widely distributed across sub-Saharan Africa and in many places throughout non arid part of Africa as compared to the cultivated eggplant species. The most widely cultivated species of the African eggplants, namely

*Solanum aethiopicum, S. macrocarpon* and *S. anguivi* species have gained widespread cultivation in African and India largely because of major improvements in the fruits sizes and shapes (Denton and Nwangburuka, 2011). These crops are commonly consumed in Tanzania, Uganda and Rwanda and the fruity forms are important component of vegetable diet, sold in grocery stores and retail outlets (Adeniji and Agatha, 2012).

The African eggplants are highly polymorphic and variable in plant structure, fruits and leaf characters. The leaves are large, hairy on the underside and alternate on the stems. Leaf prickles and hairiness are more pronounced in wild types (Jagatheeswari, 2014). The fruit of an eggplant is a fleshy berry that has colours ranging from black, white, green, shiny purple and yellow and the skin has stripes and patches. The shape of fruits varies from round to oblong, cylindrical, long and oval in shape. Anthocyanin and chlorophylls (a and b) distribution pattern controls eggplant fruit colour diversity (Frary *et al.*, 2007).

The leaves and fruits of African eggplants have bitter taste and this could be attributed to the presence of alkaloids (mainly glycoalkaloids and phenolic compounds) as described by Abukutsa-Onyango (2003). The production of alkaloids, phenolic acids and anthocyanins has led to the eggplant being used in traditional medicine (Frary *et al.*, 2007). The bitterness determines to a great extent their edibility and their use in traditional systems of medicine has been on record for a long time (Chadha and Mndiga, 2007). There is increasing evidence that intake of their leaves and fruits reduce the incidence of chronic diseases including diabetes and artherosclerosis (Kwon *et al.*, 2008; Elekofehinti *et al.*, 2012). Besides, the plants can grow in agricultural wastelands, are somewhat drought resistant and have the ability to grow in humid areas. They have also even proven to be resistant to molds, mildews and certain soil-borne plant pathogens (Sękara *et al.*, 2007). They can be grown alongside other crops or in small pots providing a high yield of fruit from a small area. Their domestication, human selection, mutation, hybridization and natural inter-crossing have resulted in expansion in fruit size, colour and shape while decreasing fruit bitterness and leaf prickliness (Frary *et al.*, 2007). Besides, like many other crops indigenous to Africa, the eggplants are easy to grow making it a good plant for research (Abukutsa-Onyango, 2003; Denton and Nwangburuka, 2011).

## 2.2 Plant response to drought stress

Stress factors have been classified as biotic and abiotic stresses. The biotic stresses are mainly due to pest and disease infection whereas abiotic stresses are contributed by inadequate water, salinity, high temperature or irradiation (Akula and Ravishankar, 2011). Drought stress is one of the major abiotic factors influencing growth and yield of crops (Jaleel *et al.*, 2009) as well as affecting their physiological and biochemical processes (Azadeh *et al.*, 2014). It leads to deterioration of thylakoid membranes, causes reduction in photosynthesis and substantial damage to photosynthetic pigments (Anjum *et al.*, 2011). In addition, it triggers an increased formation of reactive oxygen species (ROS) (Sánchez-Rodríguez *et al.*, 2012) which stimulate biosynthesis or degradation of important molecules such as proline, glycerol, sugars/carbohydrates, antioxidants, glycine betaine as well as secondary metabolites (Bartoli *et al.*, 2006; Kosar *et al.*, 2015). An imbalance between ROS and cellular antioxidants constitutes oxidative stress (Davey *et al.*, 2007), which affects antioxidant content in plants and may cause cellular damage (Miyake, 2010). Plant metabolism is a dynamic system; plants adapt to stress conditions through mechanisms associated with higher or lower expression of specific metabolites (Fischbach and Clardy, 2007). In addition, several of the necessary changes (Figure 2.1) have been linked to improved stress tolerance and may lead to some modifications in secondary chemistry in drought-stressed plants (Peñuelas and Munné-Bosch, 2005).

The Solanaceae crops particularly tomato and eggplant are well adapted to almost all climatic regions of the world. However, abiotic stresses are the primary constraint of these crop's yield potential (Gerszberg and Hnatuszko‐Konka, 2017). Molecular pathways underlying abiotic stress tolerance have recently been studied intensely with much emphasis on the tolerance mechanisms pertaining to drought stress. Unfortunately, drought stress is complex in nature and controlled by networks of different factors such as genetic and environmental that hinders crop plant breeding strategies (Da Silva and de Oliveira, 2014). In this context, a metabolomic approach is required to obtain a complete picture of the plant metabolic network during drought stress.

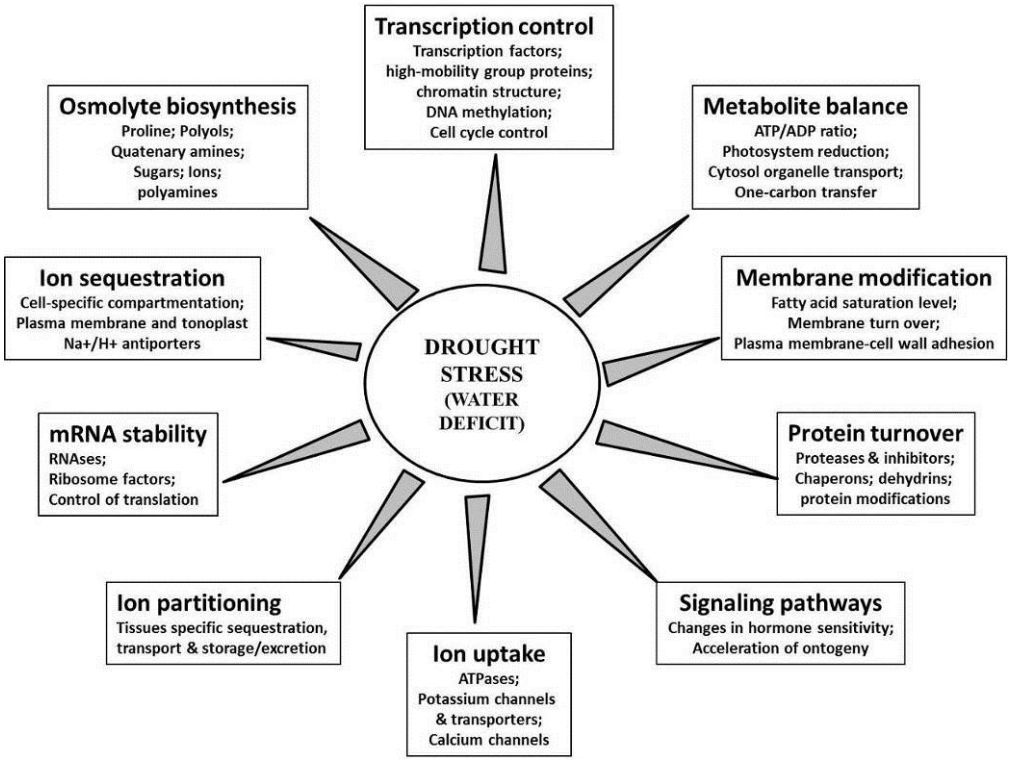



Figure 2.1: Plant responses to drought stress shows many cellular processes changes which allow the plant to maintain metabolism and restore conditions that allow for continued growth under stress. Modified from Bray (1993).

### 2.3 Functional diversity of African solanaceae

The Solanaceae family is highly diverse (Knapp *et al.*, 2004) and only few members such as potato, tomato, pepper and eggplant among others are important as food sources. Cultivated tomato and its wild relatives has been widely used as model plants, first in classical and then in cellular and molecular genetics. On the other hand, the cultivated eggplants are less suitable as models for fundamental research despite being of worldwide importance (Gebhardt, 2016). Wide morphological diversity exists in eggplants and tomatoes, their related species and the wild types. This is observed in plant morphology (inflorescence, leaf and fruit), physiology and biochemical properties (Bationo-Kando *et al.*, 2015). Fruit shape, size and color and taste are the most noticeable characters that vary among the individual crops (Frary *et al.*, 2007). These crops are susceptible to several pests and diseases, nematodes as well as abiotic stress conditions (Kashyap *et al.*, 2003). The wild relatives offer better resistance to most of the destructive pests and pathogens and are more tolerant to drought and salinity problems. This pool of genetic

variation within the African Solanaceae is the basis for selection as well as for plant improvement. This genetic diversity can be tapped to address current and future needs regarding food and nutritional security in a long term sustainable way (Schippers, 2002; Kouassi *et al.*, 2014). Thus, conservation of this plant genetic diversity is essential for present and future human well-being (Asthana and Asthana, 2012).

### 2.4 Common quality traits of African solanaceae

The African solanaceae particularly eggplant and tomato offer better resistance to most of the destructive pests and pathogens and are more tolerant to drought and salinity problems. They contain useful genes that can be exploited in genetic improvement of cultivated species. There is enormous untapped genetic diversity in African Solanaceae that can be used to address current and future needs regarding food and nutritional security in a long term sustainable way (Schippers, 2002; Kouassi *et al.*, 2014). Beside this, important secondary metabolites such as flavonoids, tannins and steroids are also found in solanaceae particularly the tomato and eggplant. Their fruits are also rich in chlorogenic acid, a polyphenol with high nutraceutical potential.

### 2.5 Common uses of solanaceae

Solanaceae species have enormous importance as food plants in the world (Samuels, 2009). They are known for their rich bioactive compounds. They are valuable nutritionally due to high content of antioxidants, including carotenoids (lycopene, β - carotene and lutein), vitamins (folate, vitamin E and C) and phenolic compounds (phenolic acids, flavonoids and tannins), lycopene, cinnamic acids, quercetin and cyanidin 3-glucoside (Kowalska and Wiercinski, 2003; Wang *et al.*, 2011). Eggplants also have considerable amount of anthocyanin, which provides for its high antioxidant value (Azuma *et al.*, 2008). These compounds are associated with several beneficial effects on human health (Raiola *et al.*, 2014). Consumption of tomato and eggplants is convincingly associated with a reduced risk of cancer and cardiovascular diseases (Canene-Adams *et al.*, 2005). Besides, eggplant fruits are characterized by low calorie content and high nutritional value (Kowalska and Wiercinski, 2003).

## 2.6 Plant metabolites

These are compounds produced by plants during the process of metabolism (Neilson *et al.*, 2013). Several important attributions for human health originate from plant metabolites including the impact on food quality and nutritional value or drug research (Fester, 2015). Most metabolites are potent antioxidants, and modifiers of cell signaling pathways and their consumption is known to confer protection against cardiovascular diseases (CVDs), cancers, diabetes and arthritis (Zhang *et al.*, 2009). The World Health Organization (WHO) estimates that 80% CVDs and one-third of cancers can be avoided by vegetable and fruit diet (WHO, 2003). Anthocyanins are one such group of nutraceuticals known to play a protective function against chronic diseases (Dai and Mumper, 2010). Increased consumer awareness of the protective functions of foods and higher incidence of CVDs and cancers in the population has increased the demand for fruits and vegetables (Miguel, 2011). Plant metabolites are divided into two categories, primary metabolites and secondary metabolites (Hong *et al.*, 2016).

### 2.6.1 Primary metabolites

Primary metabolites are compounds produced by plants in a wide range and represent the real endpoints of metabolism and any physiological underlying regulatory processes (López-Gresa *et al.*, 2010). They are essential for life and exist in all plants and include compounds such as sugars, amino acids, organic acids, lipids and tricarboxylic acid (TCA) cycle intermediates (Hong *et al.*, 2016). They are found across a huge range of concentrations and are utilized as food, industrial and energy-source materials, drugs and medicines since they contribute to major traits such as food quality, taste, nutritional value, toxicity and allergenicity (Hefferon, 2015).

The quality of food in terms of nutritional value, taste, fragrance and appearance is essentially determined by its biochemical composition (Hall, 2006). Therefore, individual primary metabolites are considered to serve as markers for health or disease status. These metabolites therefore, promote human health not only as dietary supplements but also as pharmaceuticals and functional foods (Raskin *et al.*, 2002).

### 2.6.2 Secondary metabolites

Secondary metabolites are a large, diverse array of organic compounds produced by plants. They are not directly involved in the normal life cycle and have no direct function in growth and

development of plant but help the plant adjust to the surroundings (Schafer *et al.*, 2009). Many have been suggested to have important ecological functions in plants such as providing protection against herbivores and pathogens, aiding in pollen and seed dispersal and curbing environmental changes (Moco *et al.*, 2007). They include compounds such as polyphenols, alkaloids, flavonoids and volatile organic compounds (Schoedl *et al.*, 2013).

Secondary metabolites are species-specific as they usually exhibit ecological functions and respond to particular stress conditions as antioxidants, reactive oxygen species (ROS) scavengers, coenzymes, UV and excess radiation screen and also as regulatory molecules (Arbona *et al.*, 2013). They are produced in a specific organ, tissue or cell type at specific stages of development for example during flower, fruit, seed or seedling development (Moco *et al.*, 2007). They can be present in the plant in an active state or as a prodrug (phytoalexins) that becomes activated upon wounding, infection or in the body of an herbivore (Shanker and Venkateswarlu, 2011). Their concentration in a given plant often varies. In agriculturally important species, the composition of secondary metabolites in plant tissue may affect the quality of food or foodstuff produced for humans and animals (Stobiecki *et al.*, 2002; Tugizimana *et al.*, 2013), in particular, flavonoids as a result of their antioxidant properties.

### 2.7 Metabolomic research

Metabolomics is the comprehensive qualitative and quantitative analysis of wide arrays of metabolites present in biological samples (Fiehn, 2002; Shulaev, 2006). It detects the metabolites which can be a substrate or a product of a biochemical pathway (Yoko *et al.*, 2008; Hong *et al.*, 2016) and hence is a powerful tool to gauge the functional and cellular regulation in any given species (Liu *et al.*, 2015). It has proved to be important in comprehensive profiling of plant derived samples for the study of plant systems and natural products research (Moco *et al.*, 2006). In addition, metabolomic-based strategies are already being designed for a diversity of applications in, for example, food processing and quality control, plant breeding for improved crop varieties and in the development of novel foodstuffs. Besides, it meets the requirements for the evaluation of multi-component herbal medicines *in vivo*, and therefore, bridges the gap between herbal medicine/traditional medicine and molecular pharmacology (Wang *et al.*, 2005).

Metabolic fingerprinting has been done with the most diverse techniques such as spectroscopic, chromatographic or hyphenated techniques, like GC-MS and LC-MS (Bovy *et al.*, 2007; Okazaki and Saito, 2012). Where issues of quantification and identification are important, NMR and specifically mass spectrometry have been the principle detection techniques to be used (Leiss *et al.*, 2011). This makes them ideal tools for broad-range profiling of abundant metabolites and for metabolite fingerprinting of extensive sample collections (Dixon *et al.*, 2006). The analytical instruments suited for each category are different. Generally, the GC-MS has been used specifically for profiling the primary metabolites while the LC-MS for secondary metabolites to allow for identification and quantification of the separated metabolites. The GC-MS has best suited to detect polar compounds such as organic acids, amino acids, sugars and sugars alcohols and nonpolar compounds such as fatty acids and sterols. This method offers high reproducibility of retention times and mass spectra, and reference databases.

Tomato is one of the most widely cultivated fruit crops that has served as a model for research on fleshy fruit development (Giovannoni, 2007), including metabolic studies (Carrari and Fernie, 2006). Metabolomics in tomato fruit are currently carried out by both GC-MS and high-resolution LC-MS technologies. In a different approach, GC-MS technology has been used to profile volatile compounds in a non-targeted manner (Tikunov *et al.*, 2005). A multi-step strategy has been employed that consists of spectral alignment of the GC-MS profiles, multivariate analysis of phenotypes at the level of molecular fragments and mass spectral reconstruction allowing metabolite recognition and identification (Hanhineva and Aharoni, 2010). Multivariate data analysis including hierarchical tree clustering, Principal Component Analysis (PCA) and the construction of a metabolite-metabolite correlation matrix, is used for non-targeted data treatment and the differences in the composition of volatile metabolites between the different genetic backgrounds (Hanhineva and Aharoni, 2010).

# CHAPTER THREE

# ASCORBIC ACID CONTENTS OF SELECTED AFRICAN EGGPLANT AND TOMATO ACCESSIONS SUBJECTED TO DROUGHT STRESS

## 3.1 ABSTRACT

Ascorbic acid is one of the most abundant water-soluble antioxidant compound present in plant tissues. However, little is known on the accumulation of ascorbic acid under drought stress conditions. This study aimed at analyzing the effects of drought stress on the contents of ascorbic acid in leaves and fruits of African eggplant and fruits of African tomato. Ascorbic acid extraction was done using metaphosphoric acid and analyzed using HPLC. From the study it was observed that ascorbic acid content increased considerably during growth and fruit ripening, and it was found to accumulate in mature stages (week 4) and mature red fruits. The ascorbic acid contents ranged between 3.47±0.36–10.79±0.72 mg/100g, 10.35±0.01–18.16±1.50 mg/100g and 12.02±0.44–28.49±0.20 mg/100g in the African eggplant leaves for the 0, 2 and 4 weeks stages, respectively. For fruits, the levels ranged between 2.72±0.33–8.90±0.36, 5.64±0.30–14.16±0.56, 9.90±0.18–23.09±0.32 mg/100g and 10.79±0.22–23.91±0.00, 14.62±0.83–26.09±1.77, 24.05±1.34–44.42±0.58 mg/100g for mature green, breaker, mature red stages of African eggplant and tomato fruits, respectively. On the other hand, drought stress was shown to increase the levels of ascorbic acid. The most pronounced difference was observed between the control and stressed accessions. Significantly high level ($p < 0.05$) of ascorbic acid was observed in drought stressed GBK50591 leaves (28.49±1.20mg/100g), RV100265 fruits (23.57±2.81 mg/100g) and V1035028 fruits (44.42±0.58 mg/100g). The results of the study indicate that ascorbic acid levels depend on the accession as well as the stage of growth and ripening, therefore, harvesting the fruits at mature red stage has improved nutritional quality. The initial ascorbic acid content may also be used as a parameter for predicting the stress tolerant crops.

## 3.2 INTRODUCTION

Ascorbic acid (vitamin C) is a major metabolite and principal nutrient in plants commonly considered as quality determining property in vegetables and fruits (Vijitha and Mahendran, 2010). It is the most abundant water-soluble antioxidant which serves as a cofactor for enzymes

involved in photosynthesis, hormone biosynthesis, and the regeneration of antioxidants (Gallie, 2013). In association with other components of the antioxidant system, ascorbic acid protects plants against oxidative damage resulting from stress (Gallie, 2013). In addition, it plays a significant role as a redox cofactor and catalyst in a broad array of biochemical reactions and processes (Noctor *et al.*, 2000; Pignocchi and Foyer, 2003). Despite accounting only for a small proportion of the total dry matter of plant leaves and fruits, ascorbic acid is highly significant from the nutritional point of view as a valuable food component.

African eggplants and tomatoes are traditionally consumed as both leafy and fruit vegetables in diverse ways. They are considered to have high nutritional quality, containing relatively large amounts of antioxidants particularly carotenoids and ascorbic acid (Chadha and Mndiga, 2007) as well as minerals and folate (Yang and Keding, 2009). Although they have been studied in different perspectives, variation in ascorbic acid content during plant growth has not been well defined. Despite the African eggplants and tomatoes having a dominant influence on the nutritional properties and antioxidant compounds, the environment in which plants grow and the stress to which they are subjected has significant impact on their composition and quality characters (Tembe *et al.*, 2017). Therefore, the concentration of these compounds in both leaves and fruits is highly influenced by environmental conditions (Bartoli *et al.*, 2006; Davey *et al.*, 2007) especially drought stress which has been shown to affect various physiological and biochemical processes in crops (Jaleel *et al.*, 2009).

Interestingly, African Solanaceae has been reported to thrive well in drought prone areas. Since several studies have shown that the defensive effects of plants against stress are related to natural antioxidants, it is noteworthy that the important properties of these African Solanaceae crops are due to ascorbic acid which constitutes their major defense system against ROS and free radicals (Azadeh *et al.*, 2014). Besides the importance of ascorbic acid in plant survival, it has been shown to have therapeutic properties forming part of the body defense system against ROS and free radicals (Halliwell and Gutteridge, 2007) therefore, prevent tissue damage. Epidemiological studies have shown that a high intake of ascorbic acid-rich fruits and vegetables is associated with decreased incidence of a wide range of degenerative disease including ageing, cancer, diabetes and cardiovascular diseases (Ulbricht and Basch, 2005). In addition, it plays a major

role in treatment of certain diseases such as scurvy, common cold, anemia, hemorrhagic disorders and wound healing (Basu and Dickerson, 1996). Unfortunately, animals and humans are unable to synthesize this vitamin and depend on exogenous sources which include plants as well as food supplements and pharmaceutical preparations. Given the fewer side effects with natural antioxidants over their artificial counterparts (Ulbricht and Basch, 2005), investigations on local vegetables and fruits may offer availability, affordability and acceptability as a health advantage. Based on this, these substances have obtained particular attention because of inducing protective effects on plants under abiotic stress. Therefore, increasing emphasis on the production of important antioxidants by plants has necessitated research leading to production of stress resistant or tolerant crops with health promoting properties. In addition, understanding plant responses to drought stress is of great importance and also fundamental parts for making the crops stress tolerant (Reddy *et al.,* 2004). In this perspective the current study aimed at evaluating the effect of drought stress and crop maturity on the level of ascorbic acid. Since ascorbic acid has well-known nutritional benefits to the consumer (Hancock and Viola, 2005), their improvement in crop species of agronomic interest is important.

## 3.3 MATERIALS AND METHODS

### 3.3.1 Plant material

Seeds of seventy four (74) African eggplant accessions and sixty one (61) African tomato accessions were obtained from gene banks at local and regional centers and institutes which include, Kenya Agricultural and Livestock Research Institute (KALRO), Gene Bank of Kenya and the World Vegetable Centre (AVRDC), Arusha, Tanzania. Out of the accessions, 19 African eggplant accessions and 18 African tomato accessions were selected for the study based on their morphological traits as shown in Table 3.1 and Table 3.2, respectively.

Table 3.1: Morphological characteristics of selected African eggplants from the accessions provided by AVRDC

| Acc Code | Species | Name | PG | PC | LVC | LBC | SC | LBS | FC | FS | FT |
|---|---|---|---|---|---|---|---|---|---|---|---|
| RV100343 | Sm | CN012 | Tall | abs | Green | DGr | Green | Sym | Red | Round | Smooth |
| RV100 199 | Ssp | EX-DAR | V. prostrate | abs | P+Gs | Gr | P+Gs | Sessile | White | Round | Smooth |
| RV100201 | Sa | GKK-AE-158 | Tall | prs | LGr | Green | L. Green | Asymm | Green | Oblong | Smooth |
| RV100332 | Sa | RNL187-194 | Tall | prs | Green | DGr | P+Gs | Asymm | Red | Round | Ridged |
| RV100271 | Sa | Line 87 | Prostrate | abs | LGr | Green | D.Green | Sym | Red | Round | Ridged |
| RV100445 | Ssp | S0004 | Prostrate | abs | Green | Green | Green | Asymm | Yellow | Oblong | Smooth |
| RV100333 | Sa | Sangawili | Intermediate | prs | LGr | Green | L. Green | Asymm | Red | Oval | Smooth |
| RV100259 | Sa | Line 55 | Intermediate | abs | Green | DGr | P+Gs | Sym | Orange | Round | Ridged |
| RV100265 | Sa | Line 21 | Prostrate | abs | Green | Green | Green | Asymm | Red | Round | Ridged |
| RV100273 | Sa | Line 89 | Prostrate | abs | Green | Green | P+Gs | Sym | Red | Round | Ridged |
| RV100511 | Sa | Sengerema 1 | Very Tall | abs | LGr | Green | L. Green | Asymm | Orange | Oval | Smooth |
| RV100432 | Ssp | N4 | V. prostrate | abs | Green | Green | P+Gs | Sym | Red | Round | Ridged |
| RVI00246 | Sa | Line 112 | Intermediate | abs | Green | Green | Green | Sym | Red | Oval | Ridged |
| RV100328 | Sa | Local Mali | Intermediate | abs | P+Gs | DGr | P+Gs | Sym | Green | Round | Ridged |
| RV100327 | Sa | Aubergine Blanche | Intermediate | abs | Green | Green | Green | Asymm | Red | Round | Ridged |
| RV100342 | Sa | Ofariwa'A | Intermediate | prs | LGr | DGr | P+Gs | Asymm | Red | Round | Ridged |
| RV100330 | Sa | Local Gaya | Prostrate | prs | Pp | DGr | D.Purple | Sym | Green | Round | Ridged |
| GBK050591 | Sa | Kenya | Intermediate | abs | LGr | DGr | Green | Sym | Yellow | Oblong | Smooth |
| RVI00438 | Sa | MM1308 | Tall | prs | Green | Green | Green | Assym | Red | Round | Smooth |

PG - Plant growth, PC – Princkles, LVC - Leaf vein colour, LBC - Leaf blade colour, SC - Stem colour, LBS - Leaf blade shape, FC - Fruit colour, FS - Fruit shape, FT - Fruit texture**.** Sm - *Solanum macrocarpon*, Sa - *Solanum aethiopicum*, Ssp – *Solanum species*. LGr - Light green, DGr – Dark green, Pp - Purple P+G – Purple + Green, P+Gs – Purple + Green stripes; Asymm- asymmetrical, Sym – Symmetrical, abs – absent, prs – present

Table 3.2: Morphological characteristics of selected African tomatoes from the accessions provided by AVRDC

| Acc code | Origin | SC | FC | GT | FD | PD | GS | EFC | Fruit shape | FCS | Fruit size |
|---|---|---|---|---|---|---|---|---|---|---|---|
| V1005987 | Morocco | Purple | Yellow | Indeterminate | Dense | Intermediate | Present | Red | Flattened | Round | Large |
| V1006833 | Ethiopia | Purple | Yellow | determinate | Dense | Intermediate | Present | Red | Cylindrical | angular | Large |
| V1005872 | Morocco | Purple | Yellow | Indeterminate | Intermediate | Dense | Present | Red | Elipsoid | Round | Large |
| VI005878 | Morocco | Purple | Yellow | Indeterminate | Intermediate | Dense | Present | Red | Rounded | Round | Large |
| V1002114 | Tanzania | Purple | Yellow | Indeterminate | Dense | Intermediate | Present | Red | Rounded | Round | Small |
| V1007108 | South Africa | Purple | Yellow | Indeterminate | Dense | Dense | Present | Red | Pyriform | Round | Intermediate |
| V1050580 | Kenya | Purple | Yellow | Indeterminate | Dense | Dense | Present | Yellow | Rounded | Round | Small |
| V1002112 | Madagascar | Purple | Yellow | determinate | Dense | Intermediate | Absent | Red | Rounded | Round | Intermediate |
| V1050589 | Kenya | Purple | Yellow | Indeterminate | Dense | Dense | Present | Yellow | Rounded | Round | Small |
| V1006838 | Ethiopia | Purple | Yellow | Indeterminate | Dense | Intermediate | Present | Red | Rounded | Round | Intermediate |
| V1006842 | Ethiopia | Purple | White | Indeterminate | Dense | Intermediate | Present | Yellow | Rounded | Round | Large |
| V1006826 | Ethiopia | Purple | Yellow | Indeterminate | Dense | Intermediate | Present | Red | Rounded | Round | Very large |
| V1006828 | Ethiopia | Purple | Yellow | Indeterminate | Dense | Intermediate | Present | Red | Rounded | Round | Intermediate |
| V1005874 | Morocco | Purple | Yellow | Indeterminate | Intermediate | Dense | Present | Red | Rounded | Round | Large |
| V1030380 | Mauritius | Purple | Yellow | Determinate | Dense | Intermediate | Present | Red | Heartshaped | Round | Intermediate |
| V1006892 | South Africa | Purple | Yellow | Indeterminate | Intermediate | Intermediate | Present | Red | High rounded | Round | Small |
| V1035028 | South Africa | Purple | Yellow | Determinate | Intermediate | Dense | Absent | Red | Rounded | Round | Intermediate |
| V1005875 | Morocco | Purple | Yellow | Indeterminate | Intermediate | Dense | Present | Red | Flattened | Irregular | Large |

Acc code- accession code, SC-stem colour, FC-flower colour, GT-growth type, FD-foliage density, PD-pubescence density, GS-presence of green shoulder, EFC-exterior fruit colour, FCS-fruit cross-section shape,

### 3.3.2 Experimental site and treatment

Experiment was carried out in a greenhouse at the Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya during September - December, 2014 under carefully controlled and optimal growth conditions (12 h light/12 h dark conditions; room temperature, forest soil). The seeds of the selected nineteen African eggplant and eighteen African tomato accessions were germinated in trays using peat moss germination media. The seedlings were then transplanted after four weeks of germination. The seedlings (one per pot) were grown alongside each other in 15 cm-diameter pots using forest soil and farm yard manure in the ratio 3:1. The spacing of 30 cm between plants and 50 cm between the rows was maintained and randomized complete block design with three replications was used. The experiment had two treatments; drought stress and control experiments. Irrigation was maintained before and after transplanting of the seedlings. Five days after transplanting, drought stress treatments were initiated and this was achieved by stopping irrigation for three days, thereafter irrigation was done after every 2 days with an equal amount of water to compensate stress. The wilting state was maintained throughout the experimental period. For the control treatment, continued watering with sufficient amount of water (normal irrigation) was maintained throughout keeping the soil moisture at over 90% field capacity. Removing of weeds was done manually on a regular basis.

### 3.3.3 Leaf and fruit sample collection

The leaf and fruit tissues were collected in the morning and carried using the cool box. Fresh leaves collected at different growth stages; before drought stress, 2 weeks and four weeks after stress were kept in zip block polythene bags and kept in the freezer at -20 $^0$C prior to analysis. Fruits on the other hand collected at mature green, breaker and mature red stages were chopped into small pieces while removing the seeds and then blended using a blender. The pulp was then packed in the zip block polythene bags and also stored at -20$^0$C prior to ascorbic acid analysis.

### 3.3.4 Ascorbic acid analysis

A reversed-phase HPLC method as modified by Ekinci and Kadakal (2005) and Vikram *et al.* (2005) was used for the analysis of ascorbic acid. Five grams of the sample was weighed and extracted with 0.8% metaphosphoric acid. The extract was made to 20 ml and centrifuged for 10 minutes at 100 rpm. The supernatant was filtered and diluted with 10 ml of 0.8% metaphosphoric

acid. This was then filtered using cotton wool, micro-filtered through 0.45 µ filter and 20 µL injected into the HPLC machine (Shimadzu Corp., Kyoto, Japan). HPLC analysis was done using Shimadzu UV-VIS detector 1.1 mL/min flow rate and wavelength of 266.0 nm. The mobile phase involving 0.8% metaphosphoric acid was filtered through a 0.45-µm membrane and degassed by sonication before use. Various concentrations of ascorbic acid standards were also made to make a calibration curve.

### 3.3.5 Data analysis

The concentration of ascorbic acid was calculated from the peak areas of the extracts using the formula derived from the standard curve. The data were subjected analysis of variance (ANOVA) to determine significant differences between respective drought stressed and control treatments and different growth and ripening stages. Mean separation was done by Fisher's protected least significant difference (LSD) test. The analysis was performed using GenStat discovery 14$^{th}$ Edition (Payne *et al.*, 2011) at 5% level of significance.

## 3.4 RESULTS

### 3.4.1 Ascorbic acid content of African eggplant leaves

The levels of ascorbic acid in African eggplant leaves were found to be accession- and growth-dependent (Table 3.3). The ascorbic acid contents in the leaves ranged between 3.47±0.36 – 10.79±0.72 mg/100g, 10.35±0.01 – 18.16±1.50 mg/100g and 12.02±0.44 – 28.49±0.20 mg/100g for the 0, 2 and 4 weeks stages, respectively. It is interesting to note that, these concentrations increased with growth and varied between growth stages (Table 3.3). Although there was significant increase ($p < 0.05$) in the levels of ascorbic acid during stress, there was clear variation with the levels of different accessions whereby some accessions particularly RV100332, RV100259, RV100432, RV100327, GBK50591 and RV100438 accumulated significantly higher ($p < 0.05$) ascorbic acid contents than the others. On the other hand, RV100445, RV100511, RV100246 and RV100330 accessions exhibited lower levels.

Table 3.3: Ascorbic acid concentration (mg/100g) of African eggplant leaves at different growth stages under drought stress

| | Control | | | Stressed | | |
|---|---|---|---|---|---|---|
| **Accession** | **0 weeks** | **2 weeks** | **4 weeks** | **0 weeks** | **2 weeks** | **4 weeks** |
| RV100343 | 4.71±0.43$^{d}$ | 10.47±0.51$^{c}$ | 20.71±0.63$^{b*}$ | 5.22±0.11$^{d}$ | 13.79±0.87$^{c}$ | 24.70±1.63$^{a}$ |
| RV100199 | 9.77±0.04$^{d*}$ | 14.36±0.87$^{c*}$ | 18.62±0.42$^{b}$ | 10.79±0.72$^{d*}$ | 14.03±0.95$^{c}$ | 22.62±1.07$^{a}$ |
| RV100201 | 7.50±0.30$^{d*}$ | 12.56±0.38$^{c}$ | 17.96±0.16$^{b}$ | 7.35±0.02$^{d}$ | 16.41±0.18$^{b}$ | 21.63±0.55$^{a}$ |
| RV100332 | 6.22±1.06$^{d}$ | 10.86±0.64$^{c}$ | 18.67±0.95$^{b}$ | 6.78±0.26$^{d}$ | 16.55±0.45$^{b*}$ | 27.75±1.81$^{a*}$ |
| RV100271 | 6.38±1.76$^{d}$ | 11.79±0.90$^{c}$ | 18.52±0.75$^{b}$ | 6.05±1.09$^{d}$ | 13.40±0.39$^{c}$ | 23.69±0.31$^{a}$ |
| RV100445 | 7.32±0.77$^{c*}$ | 10.89±0.04$^{b}$ | 17.90±1.47$^{a}$ | 7.95±1.03$^{c}$ | 16.89±0.30$^{a}$ | 19.18±0.42$^{a}$ |
| RV100333 | 7.48±0.08$^{c*}$ | 10.43±0.18$^{c}$ | 15.61±1.11$^{bc}$ | 8.83±0.27$^{c*}$ | 18.16±1.50$^{b*}$ | 22.65±1.51$^{a}$ |
| RV100259 | 4.96±1.15$^{d}$ | 10.60±0.32$^{c}$ | 18.26±1.39$^{b}$ | 5.58±0.61$^{d}$ | 13.69±0.55$^{c}$ | 26.93±1.04$^{a*}$ |
| RV100265 | 4.23±0.18$^{d}$ | 10.89±0.05$^{c}$ | 16.56±0.26$^{b}$ | 4.71±0.29$^{d}$ | 13.15±0.88$^{bc}$ | 23.41±0.74$^{a}$ |
| RV100273 | 5.97±1.23$^{d}$ | 10.40±0.22$^{c}$ | 20.38±3.09$^{a*}$ | 5.96±0.78$^{d}$ | 14.33±0.16$^{b}$ | 22.77±1.79$^{a}$ |
| RV100511 | 4.15±0.35$^{d}$ | 10.35±0.01$^{c}$ | 16.90±1.50$^{b}$ | 4.02±0.41$^{d}$ | 12.75±0.72$^{c}$ | 20.84±0.48$^{a}$ |
| RV100432 | 5.29±0.17$^{c}$ | 14.06±1.49$^{b*}$ | 22.14±0.54$^{a*}$ | 5.53±0.15$^{c}$ | 17.26±1.22$^{b*}$ | 25.78±1.83$^{a*}$ |
| RV100246 | 5.85±0.36$^{d}$ | 11.99±0.32$^{c}$ | 18.08±0.29$^{a}$ | 6.06±0.16$^{d}$ | 16.97±0.41$^{b*}$ | 20.06±0.84$^{a}$ |
| RV100328 | 5.09±0.36$^{d}$ | 10.67±0.28$^{c}$ | 17.16±1.41$^{b}$ | 5.83±0.17$^{d}$ | 13.73±0.41$^{c}$ | 21.05±0.63$^{a}$ |
| RV100327 | 3.74±0.15$^{c}$ | 12.53±1.03$^{b}$ | 12.02±0.44$^{b}$ | 3.58±0.26$^{c}$ | 13.73±0.51$^{b}$ | 25.71±0.66$^{a*}$ |
| RV100342 | 5.49±0.75$^{d}$ | 11.05±0.32$^{c}$ | 17.88±0.17$^{b}$ | 5.50±0.12$^{d}$ | 14.10±0.27$^{c}$ | 24.09±1.89$^{a}$ |
| RV100330 | 4.38±0.68$^{d}$ | 11.48±0.57$^{c}$ | 15.18±2.43$^{b}$ | 3.85±0.48$^{d}$ | 12.92±0.92$^{bc}$ | 20.84±0.93$^{a}$ |
| GBK50591 | 7.31±0.20$^{c*}$ | 15.02±0.68$^{b*}$ | 16.80±1.38$^{b}$ | 9.20±0.39$^{c*}$ | 15.76±0.75$^{b}$ | 28.49±1.20$^{a*}$ |
| RV100438 | 3.61±0.08$^{d}$ | 11.64±1.27$^{c}$ | 15.38±0.92$^{b}$ | 3.47±0.36$^{d}$ | 13.22±0.23$^{bc}$ | 26.24±1.19$^{a*}$ |

Mean ± SD fresh weight (n=3).
Values on the same row with different superscripts are significantly different whereas means with asterisk (*) within the column were significantly higher ($P < 0.05$)

### 3.4.2 Ascorbic acid concentration of African eggplant fruit tissues

For the African eggplant fruits, the level of ascorbic acid ranged between 2.72±0.33 – 8.90±0.36, 5.64±0.30 – 14.16±0.56 and 9.90±0.18 – 23.09±0.32 mg/100g fw for mature green, breaker and mature red, respectively (Table 3.4). Significant differences ($p < 0.05$) were observed in the amount of ascorbic acid in the different accessions of African eggplants studied (Table 3.4). It was observed that the ascorbic acid concentrations were significantly higher ($p < 0.05$) for drought stressed fruits and most importantly in the mature red fruits. The highest concentration in drought stress was found in the fruits of RV100343, RV100199, RV100265 and GBK50591 accessions. For the control, the highest was observed in RV100199 and GBK50591 accessions.

Table 3.4: Ascorbic acid concentration (mg/100g) of African eggplant fruits at different ripening stages under drought stress

| | Control | | | Stress | | |
|---|---|---|---|---|---|---|
| **Accession** | **Mature Green** | **Breaker** | **Mature red** | **Mature Green** | **Breaker** | **Mature red** |
| RV100343 | 5.25±0.31$^{d}$ | 9.00±0.67$^{c}$ | 13.38±0.31$^{b}$ | 7.27±0.16$^{c*}$ | 11.74±0.56$^{b}$ | 22.03±0.07$^{a*}$ |
| RV100199 | 6.14±0.62$^{d*}$ | 11.11±0.85$^{c*}$ | 17.86±0.42$^{b*}$ | 8.90±0.36$^{c*}$ | 14.16±0.56$^{*b}$ | 23.09±0.32$^{a*}$ |
| RV100201 | 5.88±0.24$^{d}$ | 8.65±0.20$^{c}$ | 13.64±0.74$^{a}$ | 7.42±0.02$^{c*}$ | 10.92±0.86$^{b}$ | 14.75±0.77$^{a}$ |
| RV100332 | 4.68±0.60$^{c}$ | 5.99±0.77$^{bc}$ | 11.36±0.73$^{a}$ | 6.69±0.06$^{bc}$ | 9.47±0.52$^{b}$ | 12.27±0.68$^{a}$ |
| RV100271 | 3.39±1.10$^{c}$ | 6.05±0.77$^{b}$ | 11.36±0.73$^{a}$ | 6.43±0.26$^{b}$ | 10.02±0.91$^{a}$ | 12.12±1.38$^{a}$ |
| RV100445 | 4.27±0.03$^{c}$ | 6.35±0.53$^{bc}$ | 12.76±0.15$^{a}$ | 6.54±0.02$^{bc}$ | 9.54±0.60$^{b}$ | 13.23±1.22$^{a}$ |
| RV100333 | 3.97±0.20$^{d}$ | 5.81±0.29$^{c}$ | 11.82±0.07$^{b}$ | 6.26±0.39$^{c}$ | 10.29±1.52$^{b}$ | 14.89±2.72$^{a}$ |
| RV100259 | 2.72±0.33$^{d}$ | 5.83±0.17$^{c}$ | 12.18±1.38$^{a}$ | 6.86±0.01$^{c}$ | 10.52±1.22$^{b}$ | 14.49±2.19$^{a}$ |
| RV100265 | 3.41±0.52$^{d}$ | 5.92±0.51$^{c}$ | 12.75±1.25$^{b}$ | 7.37±0.52$^{c*}$ | 14.60±0.94$^{b*}$ | 23.57±2.81$^{a*}$ |
| RV100273 | 4.52±0.20$^{c}$ | 6.39±0.48$^{c}$ | 12.36±1.13$^{a}$ | 6.53±0.25$^{c}$ | 9.97±0.10$^{b}$ | 12.82±1.79$^{a}$ |
| RV100511 | 3.32±0.45$^{c}$ | 6.28±0.42$^{b}$ | 12.84±1.33$^{a}$ | 6.34±0.08$^{b}$ | 10.36±0.61$^{ab}$ | 11.78±0.36$^{a}$ |
| RV100432 | 3.60±0.23$^{d}$ | 5.64±0.30$^{c}$ | 13.17±1.80$^{a}$ | 6.44±0.06$^{c}$ | 9.10±0.22$^{b}$ | 13.11±0.09$^{a}$ |
| RV100246 | 3.13±1.41$^{c}$ | 6.46±0.04$^{b}$ | 10.30±1.69$^{a}$ | 6.40±0.38$^{b}$ | 10.10±0.87$^{a}$ | 11.65±0.37$^{a}$ |
| RV100328 | 3.81±0.04$^{d}$ | 6.00±0.51$^{c}$ | 10.81±0.53$^{b}$ | 6.45±0.03$^{c}$ | 11.00±0.74$^{b}$ | 14.07±0.56$^{a}$ |
| RV100327 | 4.20±0.23$^{c}$ | 5.85±0.41$^{c}$ | 10.25±0.16$^{b}$ | 6.56±0.27$^{c}$ | 10.51±0.27$^{b}$ | 14.63±1.86$^{a}$ |
| RV100342 | 5.00±1.96$^{c}$ | 6.04±0.49$^{c}$ | 9.90±0.18$^{b}$ | 5.77±0.05$^{c}$ | 10.24±0.22$^{b}$ | 15.75±1.35$^{a}$ |
| RV100330 | 3.39±0.45$^{d}$ | 6.45±0.16$^{c}$ | 10.12±0.49$^{b}$ | 6.46±0.41$^{c}$ | 10.11±0.31$^{b}$ | 17.53±1.67$^{a}$ |
| GBK50591 | 7.03±0.08$^{d*}$ | 10.98±0.46$^{c*}$ | 16.25±0.55$^{b*}$ | 8.39±0.62$^{d*}$ | 13.02±0.59$^{b*}$ | 20.07±0.64$^{a*}$ |
| RV100438 | 2.79±0.92$^{d}$ | 6.21±0.26$^{c}$ | 11.75±1.75$^{b}$ | 6.52±0.30$^{c}$ | 9.59±0.72$^{b}$ | 15.95±1.97$^{a}$ |

Mean ± SD fresh weight (n=3).
Values on the same row with different superscripts are significantly different whereas means with asterisk (*) within the column were significantly higher ($P < 0.05$)

### 3.4.3 Ascorbic acid concentration in African tomato fruits

The results of the African tomato fruits showed significant differences ($p < 0.05$) in the amount of ascorbic acid in the different accessions studied. The level of ascorbic acid increased during ripening in all tissues, though its increase was generally largest between mature green and breaker stage (Table 3.5). Ascorbic acid content of the accessions varied from 10.79±0.22 - 23.91±0.00, 14.62±0.83 – 26.09±1.77 and 24.05±1.34 – 44.42±0.58 mg/100g fw for mature green, breaker and mature red, respectively. The lowest ascorbic acid levels we observed in V1006842 accession. On the other hand, the results showed the cherry type tomato accessions; V1050580 and V1050589 had generally higher ascorbic acid contents in the breaker stage than in the mature red stage. Besides the varying concentrations of ascorbic acid during ripening, the level of ascorbic acid content increased significantly ($p < 0.05$) on the drought stressed fruits as

compared to control fruits (Table 3.5). The highest concentration in was found in drought stress samples of V1007108, V102112, V1005874, V1030380, V1006892 and V1035028 tomato accession at mature red stage. The drought stressed samples of V1002114, V1050580, V1050589 and V1005875 at breaker stage also had higher levels.

Table 3.5: Ascorbic acid concentration (mg/100g) of tomato fruits at different ripening stages under drought stress

| | **Control** | | | **Stressed** | | |
|---|---|---|---|---|---|---|
| **Accession** | **Mature Green** | **Breaker** | **Mature red** | **Mature Green** | **Breaker** | **Mature red** |
| V1005987 | 12.10±0.56$^{c}$ | 17.77±0.09$^{c}$ | 27.85±0.48$^{b}$ | 15.85±0.33$^{c}$ | 29.01±2.27$^{b}$ | 37.46±0.06$^{a}$ |
| V1006833 | 15.01±0.24$^{c*}$ | 18.70±0.19$^{c}$ | 28.48±0.47$^{b}$ | 17.78±0.78$^{c}$ | 29.39±0.61$^{b}$ | 35.55±0.30$^{a}$ |
| V1005872 | 14.58±0.01$^{c}$ | 16.89±0.03$^{c}$ | 26.34±2.55$^{b}$ | 17.47±0.29$^{c}$ | 26.09±1.77$^{b}$ | 34.75±1.24$^{a}$ |
| VI005878 | 16.06±0.10$^{d*}$ | 19.95±0.47$^{d}$ | 27.77±0.73$^{bc}$ | 23.91±1.00$^{c*}$ | 31.90±0.61$^{b}$ | 40.02±7.87$^{a}$ |
| V1002114 | 10.79±0.22$^{d}$ | 24.02±0.25$^{b}$ | 15.18±0.28$^{c}$ | 17.24±0.19$^{c}$ | 40.17±0.69$^{a*}$ | 29.25±0.98$^{b}$ |
| V1007108 | 13.59±0.57$^{d}$ | 20.23±0.01$^{c}$ | 30.98±0.58$^{b}$ | 15.63±0.48$^{cd}$ | 32.57±0.12$^{b}$ | 41.32±0.66$^{a*}$ |
| V1050580 | 12.05±1.90$^{d}$ | 30.00±0.22$^{b*}$ | 19.50±0.20$^{c}$ | 22.91±1.42$^{c*}$ | 40.79±0.40$^{a*}$ | 28.26±0.85$^{b}$ |
| V1002112 | 10.82±0.25$^{d}$ | 17.91±0.54$^{c}$ | 28.72±1.58$^{b}$ | 19.51±0.15$^{c}$ | 31.54±0.57$^{b}$ | 41.26±1.02$^{a*}$ |
| V1050589 | 12.78±1.34$^{d}$ | 29.84±0.00$^{b*}$ | 20.99±0.00$^{c}$ | 17.30±2.95$^{c}$ | 39.94±3.38$^{a*}$ | 31.78±0.35$^{b}$ |
| V1006838 | 11.33±0.49$^{d}$ | 25.72±0.51$^{c}$ | 21.24±0.51$^{c}$ | 23.91±0.04$^{c*}$ | 42.52±2.92$^{a}$ | 30.78±1.08$^{b}$ |
| V1006842 | 10.82±0.15$^{b}$ | 14.62±0.83$^{b}$ | 24.16±0.57$^{a}$ | 13.21±1.51$^{b}$ | 23.35±0.00$^{a}$ | 26.32±1.24$^{a}$ |
| V1006826 | 10.82±0.92$^{d}$ | 20.13±0.29$^{c}$ | 29.20±0.54$^{b}$ | 20.30±0.96$^{c}$ | 29.78±0.61$^{b}$ | 40.87±1.07$^{a}$ |
| V1006828 | 14.00±0.88$^{d}$ | 18.97±0.59$^{c}$ | 27.40±0.57$^{b}$ | 19.82±0.89$^{c}$ | 28.26±0.44$^{b}$ | 39.89±1.13$^{a}$ |
| V1005874 | 11.94±2.58$^{d}$ | 17.90±0.10$^{dc}$ | 30.61±0.59$^{b}$ | 22.50±0.57$^{c}$ | 29.45±1.23$^{b}$ | 41.27±0.62$^{a*}$ |
| V1030380 | 15.87±0.42$^{d*}$ | 18.51±0.67$^{c}$ | 36.56±0.12$^{b*}$ | 20.96±0.72$^{c}$ | 32.61±0.59$^{b}$ | 42.73±0.42$^{a*}$ |
| V1006892 | 13.98±2.09$^{d}$ | 15.23±0.65$^{d}$ | 24.72±0.67$^{c}$ | 21.53±1.81$^{c}$ | 30.72±3.71$^{b}$ | 42.35±0.65$^{a*}$ |
| V1035028 | 14.33±1.35$^{d}$ | 18.38±0.85$^{cd}$ | 24.05±1.34$^{c}$ | 19.90±0.70$^{cd}$ | 30.51±1.97$^{b}$ | 44.42±0.58$^{a*}$ |
| V1005875 | 15.15±0.99$^{c*}$ | 30.48±0.91$^{b*}$ | 18.24±0.58$^{c}$ | 20.44±2.45$^{c}$ | 42.24±1.43$^{a*}$ | 29.12±0.28$^{b}$ |

Mean ± SD fresh weight (n=3).
Values on the same row with different superscripts are significantly different whereas means with asterisk (*) within the column were significantly higher ($P < 0.05$)

## 3.5 DISCUSSION

Drought is the major limiting factor that affects plant growth and yield (Shao *et al.,* 2007) as well as many physiological and biochemical processes in plants (Reddy *et al.,* 2004). It leads to oxidative stress in plants, enhancing the generation of ROS (Sánchez-Rodríguez *et al.*, 2012). However, efficient destruction of ROS in plant cells requires the concerted action of antioxidants including ascorbic acid which is required for many key metabolic functions in plant cells (Foyer *et al.*, 2012). A high level of ascorbic acid is essential to effectively maintain the antioxidant

system that protects plants from oxidative damage due to the biotic and abiotic stresses (Reddy *et al.*, 2004).

In this study, African eggplant and tomato plants were subjected to drought stress to evaluate its effects on ascorbic acid in leaves and fruits. The degree to which the ascorbic acid concentrations changed under drought stress was extremely variable among different accessions and different growth stages. For the eggplant leaves, the results showed that the amount of ascorbic acid increased with its age. A significantly low concentration ($p < 0.05$) was observed in young leaves whereas older leaves (4 weeks) had high levels. These results agree with other studies which reported increased ascorbic acid content with plant age (Stino *et al.*, 1973; Gallie, 2013). In other leafy vegetables, ascorbic acid contents have been found to change during growth, but with no consistent increase or decrease (Miyajima, 1994; Gallie, 2013). The rate of deterioration of ascorbic acid has often been related to metabolic and respiration rate, which is usually higher in younger leaves (Bergquist *et al.*, 2006). This may therefore explain the higher ascorbic acid accumulation in the mature leaves as evident in the findings of this study.

Besides the change in the concentration of ascorbic acid during growth of the eggplants, drought stress also affected the concentration. For specific responses, a significant drought effect was observed in leaves of some accessions than others and this implies that the different accessions mentioned have varying contributions. It is also notable that accession RV100438, GBK50591, RV100332 and RV100259 were repeatedly among the accessions with the highest concentration of ascorbic acid. These accessions were characterized by their unique morphological characteristics such as presence of prickles and this might have contributed to high contents although no association has been observed between the prickles and the ascorbic acid content. Similarly, significant increase ($p < 0.05$) in ascorbic acid of RV100342, RV100438 and RV100343 accessions was observed in drought stressed accessions as compared to control accessions at mature red stages. Therefore, the drought stressed plants had significantly higher ($p < 0.05$) ascorbic acid level as compared to the control. This is consistent with other studies and reviews which have mainly reported increased or unchanged ascorbic acid content in leafy vegetables (Tambussi *et al.*, 2000).

The ascorbic acid contents of African eggplant and tomato fruits exhibited similar trends as the eggplant leaves. For the African eggplant fruits, the levels of ascorbic acid increased as the fruit ripened and the mature red fruits had higher levels as compared to the mature green stages. Although there was similar trend for the ascorbic acid levels of both the African eggplant leaves and fruits, the concentrations of the leaves were higher as compared to the fruits. The highest concentration was found in the fruits of some accessions, particularly RV100343, RV100199 and GBK50591 accessions may be attributed to the variations in the morphologies (SanJosé *et al.*, 2014). Previous studies have mainly reported similar findings in different tomato accessions with ascorbic acid contents ranging between 10.86 – 18.56 mg/100g fw (Pinela *et al.*, 2012), 19.77 – 37.80 mg/100g fw (Dar and Sharma, 2011), 9.70 – 18.00 mg/100g (Frenich *et al.*, 2005) and 146.56±2.51 – 1670.74±7.61 µg/g (Moco *et al.*, 2007). In addition, the values were comparable to those reported for other fruits known as sources of ascorbic acid such as apples (1.1 - 3.5 mg/100g fresh samples), grapefruits (28.5 - 52.0 mg/100g fresh samples), mangoes (9.1 - 18.6 mg/100g fresh samples) and oranges (42.1 - 62.4 mg/ 100g) (Franke *et al.*, 2004). According to SanJosé *et al.* (2014) and Tembe *et al.* (2017), the ascorbic acid levels of eggplant and tomato fruits are influenced by genotype of the plant. On the other hand, the ascorbic acid of the tomato accessions evaluated varied with the ripening stages. This is in agreement with several studies which have reported that, ascorbic acid content significantly increase during fruit maturation and ripening (Davey *et al.*, 2007; Gautier *et al.*, 2008). Comparing the ripening stages of different accessions, the mature red fruits had a higher ascorbic acid level than the breaker and mature green fruits. Contrary to the African eggplant, the cherry type tomato accessions (with smaller fruits) had generally higher ascorbic acid contents. The breaker stage of some cherry type accessions such as V1005875, V1050580, V1050589, V1006838 and V1002114 reported significantly higher ($p < 0.05$) ascorbic acid content as compared to the mature red.

In addition, the results on the ascorbic acid content were in agreement with other findings which have reported that the values of ascorbic acid vary with the cultivars and accessions (Gallie, 2013). Similarly, ascorbic acid prevents oxidative damage that takes place during fruit ripening, thus maintaining the shelf life of the fruit (Davey *et al.*, 2007). Moreover, from the study, the ripening of tomato fruits was dependent on the accessions where some accessions ripened earlier than the others. The accessions which ripened at a faster rate were shown to contain higher

amounts of ascorbic acid as compared to those that ripened at a relatively slower rate. In addition, the results show cherry type tomato accessions (with smaller fruits) had generally higher ascorbic acid contents. On the other hand, the yellow-fruited cherry type accessions V1050580 and V1050589 reported higher ascorbic acid levels in the breaker stage as compared to the mature red fruits. The results reflect a range of genetic factors that contributes to quantitative variation across varieties. According to the studies by Tembe *et al.* (2017), they observed that GBK 050580 accession reported lowest reduction in fruit weight during drought stress. Likewise, these results are in agreement with those of Adalid *et al.* (2010) and Vinkovic-Vrcek *et al.* (2011) who concluded that smaller fruits (cherry type tomatoes) have generally higher vitamin C content. This suggests that ascorbic acid accumulation in cherry tomatoes may be caused by a combination of increased metabolic flux and reduced utilization of ascorbic as observed by Di Matteo *et al.* (2010). Therefore, the increase in ascorbic acid concentration in the African eggplant and tomato leaves and fruits with growth and ripening stages demonstrates the nutritional importance of these crops.

Drought stress has been reported to induce oxidative stress in plants (Davey *et al.,* 2007; Boyer *et al.,* 2008). In this study, drought was also observed to affect the ascorbic acid contents of African eggplant and tomato fruits. Under water stress conditions, ascorbic acid assist in the counteraction of the adverse effects of water stress, stabilization and protection of the photosynthetic pigments and the photosynthetic apparatus from oxidization (Khan *et al.*, 2011). In agreement with the results of this study, earlier reports (Dumas *et al.*, 2003; Gautier *et al.*, 2008), have shown positive effects of stress conditions on ascorbic acid levels in plants. These studies have reported ascorbic acid as an important constituent of tomato fruits (Gautier *et al.*, 2008) and that its production is promoted by water limitation in tomato (Dumas *et al.*, 2003). An experiment by Nahar *et al.* (2011) with tomato genotypes showed that concentration of ascorbic acid increased significantly under drought stress. In addition, it has been observed that plant tolerance to environmental stresses is positively correlated with ascorbic acid content (Tambussi *et al.*, 2000) and water stress caused changes in ascorbic acid concentrations in tomato fruits (Murshed *et al.,* 2008). Although in the present study the ascorbic acid levels of the stressed accessions varied among the accessions, Chaves *et al.* (2003) showed that the reactions to water stress differed significantly depending on the cultivar and stage of development. The effect of

drought stress on the ascorbic acid content during ripening is well discussed by studies by Davey *et al.* (2007) and Gautier *et al.* (2008). It is noteworthy that from the study, the increase in ascorbic acid concentration in the leaves and fruits suggests an important role for this antioxidant against oxidative stress provoked by drought stress. Therefore, these findings provide evidence that can contribute to abiotic stress tolerance in these crops. Since ascorbic acid exists as a prevalent antioxidant, its accumulation in fruits improves its quality as described by Malacrida *et al.* (2006). According to Davey *et al.* (2007), ascorbic acid levels have been linked to improvement of shelf life in apple and this may also apply to other crops. Therefore improving the antioxidant content of fruits might improve fruit quality during ripening.

Besides the importance of ascorbic acid to plants, epidemiological studies have shown a high intake of fruits and vegetables being correlated with a low incidence of a number of chronic diseases (Hung *et al.*, 2004; Ulbricht and Basch, 2005). This might be partly due to ascorbic acid (Halliwell and Gutteridge, 2007). From a nutritional point of view, crops grown under less water may be preferred due to the high concentrations of ascorbic acid. Therefore the various concentrations reported by the different accessions might be used as a parameter for predicting the drought tolerant crops. In this case, better accessions in terms of drought tolerance could be evaluated to explore the high content of ascorbic acid under drought stress. Furthermore, high accumulation of antioxidants particularly ascorbic acid may indicate favorable traits into agricultural preferred varieties. These could be logically used to breed for varieties that are drought susceptible.

### 3.6 CONCLUSION

This study revealed a wide variation in the ascorbic acid levels of the African eggplant and tomato accessions analyzed. From the current study, the ascorbic acid content was shown to vary with accession, drought stress condition, growth and ripening stages. With the increase in the ascorbic acid in mature leaves of stressed plants compared to control, it is evident that as the plant grows the level of ascorbic acid increases. African tomato fruits had high levels of ascorbic, as compared to the African eggplant fruits. A strong positive association was observed between ascorbic acid and drought stress in the different growth and ripening stages. From the results, these RV100343, RV100199, RV100265 and GBK50591 African eggplant accessions

and V1007108, V102112, V1005874, V1030380, V1006892 and V1035028 African tomato accession at mature red stage were found to be important in accumulating ascorbic acid. From the results it is evident that, harvesting the fruits at mature red stage has considerable levels of ascorbic acid content thus improved nutritional quality. Since ascorbic acid also increased with drought stress, its contents may also be used as a parameter for predicting the stress tolerant crops.

# CHAPTER FOUR

# CAROTENOID PROFILING OF SELECTED AFRICAN EGGPLANT AND TOMATO ACCESSIONS SUBJECTED TO DROUGHT STRESS

## 4.1 ABSTRACT

Carotenoids act as accessory pigments for photosynthesis and precursor to plant hormones. Though African solanaceae crops are known to adapt to various abiotic stresses, limited information is available on the effect of drought stresses on carotenoids. The study aimed at evaluating the effect of drought stress on carotenoid profiles of African eggplant leaves and fruits and African tomato fruits. The carotenoids were determined using a Dionex HPLC-PDA and Chromeleon software package. Major carotenoids such as xanthophylls (neoxanthin, violaxanthin, zeaxanthin and lutein) and carotenes (β–carotene and α–carotene), phytofluene, lycopene, phytoene together with photosynthetic pigments, chlorophylls (chlorophyll- a and b) were targeted. Lycopene and zeaxanthin were not detected in the leaf and fruits tissues, respectively. The results indicated an increased carotenoid content with maturity stage of the crop as well as the ripening stages. The total carotenoid contents varied from 1.07±0.16–23.21±4.61 mg/100g and 1.89±0.88–108.14±1.90 mg/100g for the African eggplant and tomato fruit tissues, respectively. Beta carotene was observed to be dependent on accession with RV100445 and GBK50591 reporting a significantly elevated ($p < 0.05$) level of β-carotene (15.28±1.73 and 12.80±0.39 mg/100g, respectively). Lycopene was significantly high ($p < 0.05$) in the mature red fruits and accounted for 65–92% of the total carotenoids whereas chlorophylls were high in the mature green stages. The results also showed that drought stress affects the levels of beta carotene, lycopene and chlorophylls. The chlorophylls reduced with stress in leaf and fruit tissues, beta carotene reduced in the leaves and increased in the fruits whereas lycopene increased with stress. Results in this study demonstrate that the ripe African eggplant and tomato fruits are good source of antioxidant carotenoids.

## 4.2 INTRODUCTION

Despite the importance of many indigenous plants, stress has been reported as a major limiting factor leading to change in their growth and development thus disrupting metabolic homeostasis.

Some indigenous crop species such as African eggplants and tomatoes have been reported to be adapted to diverse abiotic stresses. They are reported to utilize various defense and adaptation mechanisms against both abiotic and biotic stressing agents (López-Gresa *et al.*, 2010) by producing metabolites involved in stress responses (Tuteja and Sopory, 2008). Among these metabolites of interest are the carotenoids which act as plant accessory pigments and are widely distributed in nature (Lu and Li, 2008).

Carotenoids represent a diverse group of secondary metabolites which participate in a wide range of physiological processes, including plant growth, development, and responses to environmental stimuli (Liu *et al.*, 2015). They are important for photosynthesis and as precursors to plant hormones (Cazzonelli, 2011) since they are ancillary light pigments, photo-protectors and basic units of the photosynthetic apparatus (Fanciullino *et al.*, 2013). Besides, they are essential components of human diets and play an important role in human nutrition and medicinal purposes hence beneficial to human health (Briskin, 2000; Rao and Rao, 2007). In addition, carotenoids are potent antioxidants and free radical scavengers (Grassmann *et al.*, 2002; Deák *et al.*, 2015) in humans and are essential in evaluating the nutritional quality of the fruits (Raffo *et al.*, 2006; Gautier *et al.*, 2008). The antioxidant potential of carotenoids is of particular significance to human health, due to the fact that losing antioxidant-ROS balance results in oxidative stress, a critical factor of the pathogenic processes of various chronic disorders. Epidemiological and clinical studies have shown that intake of carotenoids is associated with a reduced prevalence of chronic degenerative diseases, including cancers, cardiovascular disorders and age-related macular degeneration (Rivera and Canela-Garayoa, 2012). This is believed to contribute to their ability to modulate the pathogenesis of cancers (Cazzonelli, 2011; Fassett and Coombes, 2011). Beta carotene is the dietary precursor of vitamin A, a well-known carotenoid derivative with widespread biological functions (Krinsky and Johnson, 2005) whereas xanthophylls (oxygenated carotenoids) are dietary antioxidants and have important preventive effects against degenerative eye diseases (Wolfgang *et al.*, 2009). On the other hand, diet containing lycopene has been associated with a decreased incidence of cardiovascular disease (Fassett and Coombes, 2011), cancer (Giovannucci, 2002; Ford and Erdman, 2012) and diabetes (Facchini *et al.*, 2000); therefore, consumption of these fruits may be of health importance.

Due to importance of carotenoids in diet and health benefits, they have been extensively studied in different matrices to analyze their distribution and levels in plants. It has been observed that carotenoids are involved in plant defense mechanism against abiotic stress (Lushchak, 2011). Specific environmental factors associated with their development have been investigated. Drought stress has been shown to generate oxidative stress in plants, leading to increased production of carotenoids in tomatoes (Dumas *et al.*, 2003). Besides the importance of carotenoids as pigments and in plant stress tolerance for adaptation, they are also the precursors of many important volatile flavor compounds in plants (Klee and Giovannoni, 2011), conferring the sensory attribute that can be detected by consumers (Vogel *et al.*, 2010). Based on the positive effect of carotenoids to plants and in the human diet, it has triggered numerous attempts to engineer plant products with enhanced carotenoid accumulation, which is not only of agricultural importance but also of scientific interest in terms of chemical, biological, and genetic regulation. Although research has been carried out on the effect of various stresses on carotenoids, the results are not always conclusive (Lumpkin, 2005). Therefore based on this, the metabolic adjustment in response to the drought stress conditions was analyzed and this highlighted carotenoids that play important roles in metabolism and physiology of the plant.

## 4.3 MATERIALS AND METHODS

### 4.3.1 Experimental site and treatments

Experiment was carried out in a greenhouse at the Boyce Thomson Institute for Plant Research (BTI), Cornell University, USA during March - May, 2015 under carefully controlled standard growth conditions (16 h light/8 h dark conditions; 26°C day, 20°C night; Cornell mix soil). The seeds of the selected nineteen African eggplant accessions (Table 3.1) were germinated in trays and the seedlings transplanted after four weeks of germination. Irrigation was maintained before transplanting and five days after transplanting of the seedlings to keep the soil moisture at over 90% field capacity. The seedlings (one per pot) were grown in 15 cm-diameter pots containing Cornel mix growth media using randomized complete block design with three replications. The spacing of 30 cm between plants and 50 cm between the rows was maintained and randomized complete block design with three replications was used. The experiment had two treatments; drought stress and control experiments. Drought stress treatments were initiated after five days of transplanting. This was achieved by stopping irrigation for two days and soil moisture monitored

every day using Delmhorst model KS-D1 Digital Soil Moisture Tester (Delmhorst Instrument Co., Towaco, New Jersey, USA). The wilting state of the crops was maintained and losses in soil moisture below 60% represented transpiration and evaporation. Thereafter irrigation was done after every 2 days with an equal amount of water (approximately 1 liter) to compensate for the loss in soil moisture. For the control treatment, continued watering with sufficient amount of water (normal irrigation) was maintained throughout the experimental period.

### 4.3.2 Sample collection

#### 4.3.2.1 Leaf sampling

Fresh African eggplant leaves were sampled at three growth stages; before stress, two weeks and four weeks after stress. The materials were harvested early in the morning from equivalent fully expanded leaves at each of the three growth stages. The harvested leaf tissues were immediately plunged (snap-frozen) in liquid nitrogen to stop further metabolism. Afterwards, they were ground in liquid nitrogen and stored in 15 mL falcon tubes at -80$^0$ C. The frozen leaf tissues were later used for carotenoid and metabolite analysis.

#### 4.3.2.2 Fruit sampling

The African eggplant and tomato fruits were collected early in the morning at different ripening stages; mature green, breaker and mature red considering the days after anthesis. Three fruits per crop were harvested and left on the bench for 3 hours. Thereafter, they were cut into small pieces while removing the seeds and immediately plunged (snap-frozen) in liquid nitrogen to stop further metabolism. The frozen tissues were then ground using a mechanical grinder in liquid nitrogen and stored as in section 4.3.2.1.

### 4.3.3 Carotenoid extraction

Carotenoids were extracted from the frozen leaf tissues using a modified protocol from Alba *et al.* (2005). About 200 mg of each tissue was weighed into 2 mL Eppendorf tubes and 2 beads added. Fifty microliters (50 µL) of 3 mg/ml magnesium carbonate suspension was added to each tube and 300 µL of tetrahydrofuran (THF) added to each tube. The mixture was homogenized in FastPrep machine (FastPrep-24, MP Biomedicals, USA) (45 seconds, speed = 5.0) and incubated at 4 $^0$C (in ice) for 20 minutes in darkness. Three hundred microliters (300 µL) of methanol was

also added and homogenized then incubated at 4 $^0$C for 10 minutes. The homogenate was then transferred to Spin-X filter, centrifuged for 1 min at 4,000 rpm (4 $^0$C). One hundred and fifty microliters (150 µL) of THF and 150 µL of methanol were added to original extraction tube and vortexed. One milliliter (1 mL) pipettor (cut tip) was used to transfer all THF/methanol/debris to spin-X filter and centrifuged again. The filtered extract was then transferred to new 2 mL tube and 450 µL of THF added to debris pellet in spin-X filter and incubated on ice for 15 minutes (dark); centrifuged for 5 minutes at maximum speed. The filtered extracts were combined and 375 µL petroleum ether and 150 µL of 25% NaCl were added to each combined extract and vortexed vigorously. It was then centrifuged at maximum speed at 4 $^0$C for 3 minutes to separate phases and the upper phase transferred to new 2 mL tube. The interphase/lower phase was re-extracted with 500 µL petroleum ether and the upper phases removed and combined with like samples. The petroleum ether extract was then rotor evaporated for 20 minutes at 45 $^0$C (to near dryness). When HPLC was not conducted immediately, the dried extracts were stored under nitrogen ($N_2$) at -80 $^0$C (dark). Five hundred microliters (500 µL) ethyl acetate was added and incubated at room temperature (RT) for 15 minutes to resuspend carotenoids; vortexed well and carotenoid suspension filtered through 0.45 µm nylon syringe filter (Cameo 3N syringe filter, GE Water and Process Technologies, USA). all the reagents were purchased as shown in Appendix 1.

### 4.3.4 HPLC analysis (YMC C30 column)

Carotenoid analysis was carried out using a HPLC machine (Dionex, ThermoFisher Scientific, USA), P680 HPLC pump, ASI-100 Automated Sample Injector, photo array detector and Chromeleon (v6.40) software package. Carotenoids were separated with polar to non-polar solvents with elution gradient (0–5 min 100% methanol:0.1% ammonium acetate; 6–25 min ramp to 4% methanol:0.1% ammonium acetate and 96% methyl t-butyl ether; 26–30 min ramp to 100% methanol:0.1% ammonium acetate; 31–35 min 100% methanol:0.1% ammonium acetate) through a guard cartridge (YMC Carotenoid S-5, 4.0 mm × 20 mm DC guard; Waters), C30 column (YMC Carotenoid S-5, 4.6 mm × 250 mm; Waters) assembly. Five channels were used for data acquisition: channel 1 (286 nm); channel 2 (348 nm); channel 3 (434 nm); channel 4 (450 nm) and channel 5 (471 nm).

### 4.3.5 Identification and quantification of carotenoids

Peak identification was performed as described in Alba *et al.* (2005). The carotenoids were identified by comparing their retention time and spectra with respective authentic standards analyzed under identical analytical conditions. Peak areas of each of the standards were used to draw the standard curve and quantify the carotenoids.

### 4.3.6 Data Analysis

The data were subjected to the statistical analysis of the variance (ANOVA) to evaluate significant differences between the accessions, different growth or ripening stages and treatments (control and drought treatments) of African eggplants and tomatoes. The analysis was performed using GenStat discovery 14$^{th}$ Edition (Payne *et al.*, 2011) at 5% level of significance. Mean separation was done by Fisher's protected least significant difference (LSD).

## 4.4 RESULTS

The difference in plant morphology under stress is illustrated in Plate 4.1 and it demonstrates how the leaves were affected by drought stress.

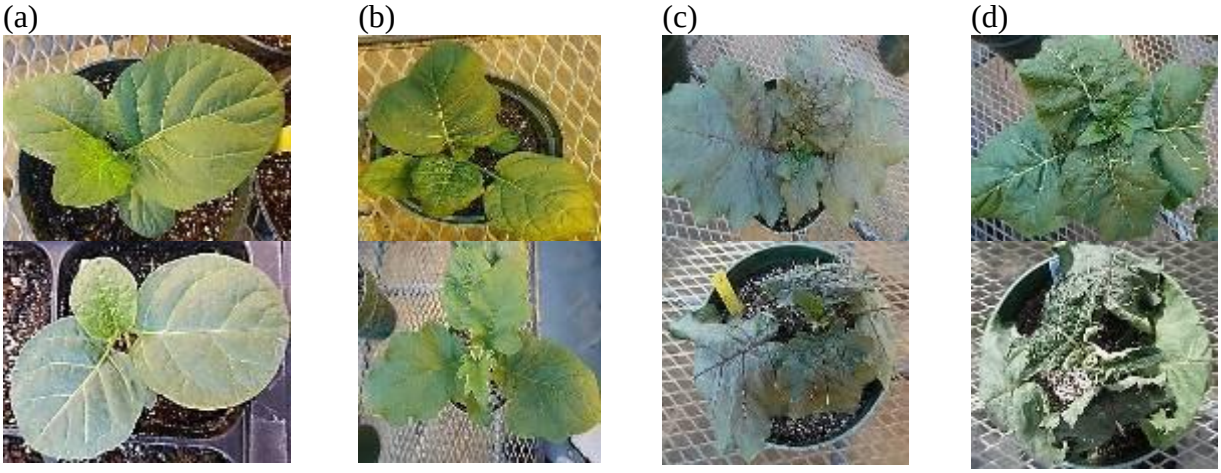



Plate 4.1: African eggplant (RV100332) accession. The top shows the control treatments of the Africa eggplant accession whereas the bottom shows the drought stress treatments of the accession (a) immediately after transplanting (b) five days after transplanting (before stress) (c) 2 weeks after stress (d) 4 weeks after stress.

| Control | | | Drought stress | | |
|---|---|---|---|---|---|
| **Mature green** | **Breaker** | **Mature red** | **Mature green** | **Breaker** | **Mature red** |
| RV100343 | | | | | |
| RV100265 | | | | | |
| RV100432 | | | | | |
| RV100246 | | | | | |
| RV100327 | | | | | |
| GBK50591 | | | | | |
| RV100330 | | | | | |

Plate 4.2: Selected African eggplant fruits at different ripening stages; mature green, breaker and mature red. The different stages are characterized by change in colour from green to red or yellow

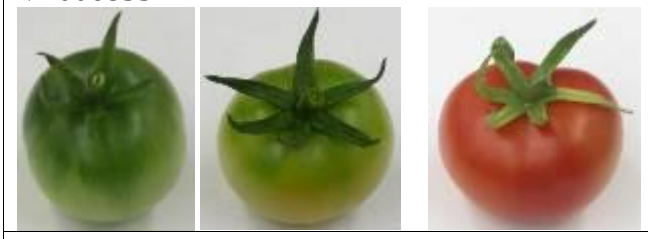
| | Control | | | Drought stressed | | |
|---|---|---|---|---|---|---|
| | **Mature green** | **Breaker** | **Mature red** | **Mature green** | **Breaker** | **Mature red** |
| V1005987 |  | | |  | | |
| V1006833 |  | | |  | | |
| V1005872 |  | | |  | | |
| VI005878 |  | | |  | | |
| V1002114 |  | | |  | | |
| V1002112 |  | | |  | | |

Plate 4.3: The African tomato fruits at different ripening stages; mature green, breaker and mature red

| | Control | | | Drought stressed | | |
|---|---|---|---|---|---|---|
| | **Mature green** | **Breaker** | **Mature red** | **Mature green** | **Breaker** | **Mature red** |
| V1050589 |  | | |  | | |
| V1006838 |  | | |  | | |
| V1006826 |  | | |  | | |
| V1006828 |  | | |  | | |
| V1030380 |  | | |  | | |
| V1035028 |  | | |  | | |

Plate 4.3: The African tomato fruits at different ripening stages; mature green, breaker and mature red

| Control | | | Drought stressed | | |
|---|---|---|---|---|---|
| **Mature green** | **Breaker** | **Mature red** | **Mature green** | **Breaker** | **Mature red** |
| V1050580 | | | | | |
|  |  |  |  |  |  |
| V1006842 | | | | | |
|  |  |  | | | |
| V1006892 | | | | | |
|  |  |  | | | |
| V1007108 | | | | | |
|  |  |  | | | |

Plate 4.3: The African tomato fruits at different ripening stages; mature green, breaker and mature red

### 4.4.1 Identified carotenoids in the African eggplant leaves and fruits and tomato fruits

The HPLC fingerprinting of the leaf and fruit extracts revealed the presence of various carotenoids as shown in spectra (Figure 4.1). The identified carotenoids included neoxanthin, violaxanthin, zeaxanthin, α-carotene, β-carotene, lutein, phytoene, phytofluene, lycopene and other unknowns. Other photosynthetic compounds such as chlorophylls (chlorophyll a and chlorophyll b) were also identified (Table 4.1). Lycopene and zeaxanthin were not detected in the leaf and fruit tissues, respectively. The concentration of the different carotenoids varied between the different accessions.

Table 4.1: Carotenoids separated on a reverse-phase C30 HPLC system and spectral characteristics used in identification from photodiode array detection

| **Carotenoid** | **Spectral characteristics (nm $\lambda_{max}$)** | **Retention time (min)** |
|---|---|---|
| Neoxanthin | 466 | 6.99 |
| Violaxanthin | 433 | 7.68 |
| Chlorophyll b | 465, 471 | 10.6 |
| Phytoene 1 | 284-286 | ~10.8 |
| Lutein | 442, 450 | ~11 |
| Chlorophyll a | 430 | 12.73 |
| Zeaxanthin | 430 | 13.33 |
| Phytoene 2 | 284-286 | ~13.5 |
| Phytofluene 1 | 343/348 | ~14.3 |
| Phytofluene 2 | 343 | ~15.1 |
| Others (unknown) | 441, 443, 464 | 15.96, 16.44, 20.7 |
| α – Carotene | 407 | 17.03 |
| β – carotene | 450 | ~17.1, 17.6 |
| Cis-Lycopene | 471 | ~22.25 |
| Trans-Lycopene | 471 | ~24 |

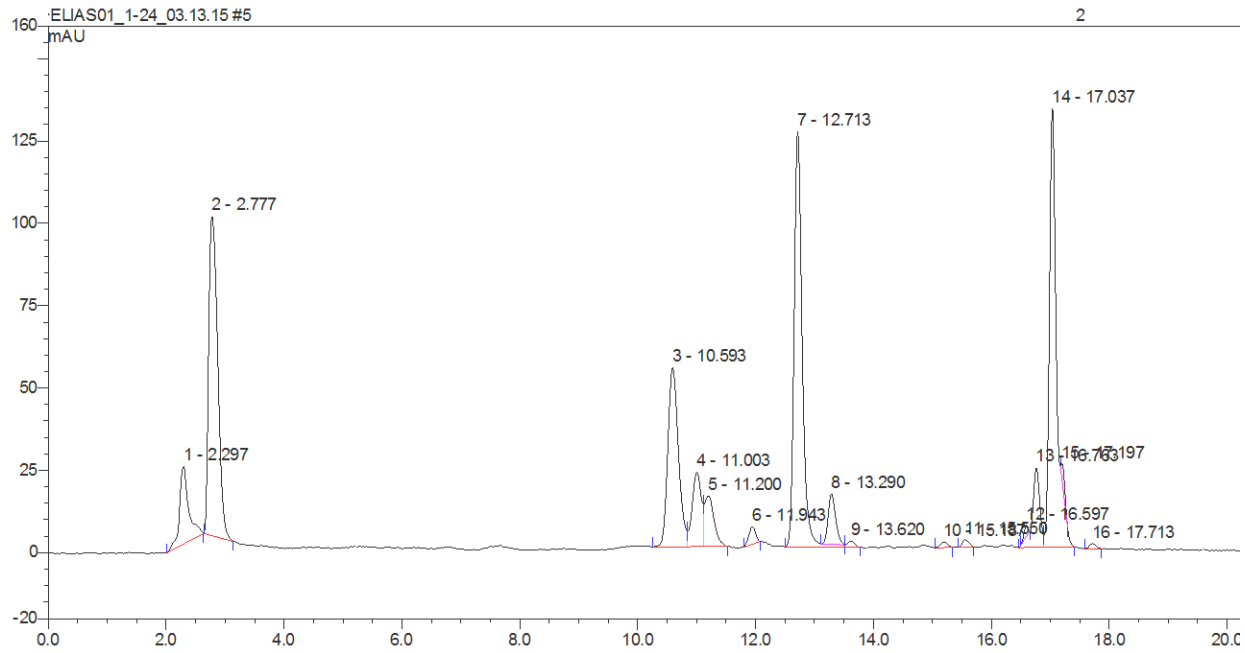



Figure 4.1: High performance liquid chromatographic profile of carotenoids in the African eggplant leaf extracts. Chlorophyll b (Rt = 10.59 min), Phytoene 1 (Rt = 11.003 min), Lutein ((Rt = 11.2 min), Xanthophyll (Rt=11.943 min), Chlorophyll a (Rt = 12.713 min), Zeaxanthin (Rt=13.29 min), Phytoene 2 (Rt=13.62 min), α - carotene (Rt=16.76 min), β - carotene (Rt=17.037 min)

### 4.4.2 Carotenoids profile of African eggplant leaves

In this study, a comparative analysis of the carotenoid and chlorophyll content of leaves of 19 African eggplant accessions at different stages of maturity under adequate water availability or drought stress conditions was done. The total carotenoids of leaves of the selected African eggplant accessions subjected to drought stress and controls are reported in Table 4.2. Maturity in the leaves reflected enhanced carotenoid metabolic activities occurring during plant growth and development. This is reported by results showing changing concentrations with progressive plant growth as well as differences between the stressed and control crops. The concentration of all the carotenoids increased during the growth of almost all the African eggplant accessions (Table 4.2). This was evident in the leaves sampled after four weeks reporting significantly higher contents ($p < 0.05$) as compared to other stages. On the other hand, there were significant differences ($p < 0.05$) in carotenoid content among the accessions used in the study. The highest of the estimated carotenoids in mature leaves was reported in RV100327 (6.84±0.18 mg/100g FW), RV100343 (5.88±0.19 mg/100g FW), RV100438 (5.66±0.12 mg/100g FW) and RV100511 (4.94±0.19 mg/100g FW) stressed accessions whereas for the control RV100327 (5.38±0.16

mg/100g), RV100332 (4.95±0.22 mg/100g FW), RV100343 (4.85±0.14 mg/100g FW), and RV100438 (4.73±0.26 mg/100g) accessions had considerable high concentration of the total carotenoids. Unlike the other accessions, the concentration of the total carotenoids for GBK50591, RV100271, RV100265, RV100511, RV100330 and RV100199 significantly increased ($p < 0.05$) in stressed crops as compared to the controls. Contrary to the trend for the other accessions, RV100201 and RV100342 reported significantly higher ($p < 0.05$) carotenoids at 2 weeks then the content reduced at week 4. However, some carotenoids were markedly characteristic of some accessions and this may be associated to their characteristic morphology for example, RV100343 which had small leaf size had higher contents as compared to the others.

Table 4.2: The total carotenoid concentration (mg/100g) in the leaves of African eggplant subjected to drought stress at different growth stages

| | **Stress** | | | **Control** | | |
|---|---|---|---|---|---|---|
| **Accessions** | **0 weeks** | **2 weeks** | **4 weeks** | **0 weeks** | **2 weeks** | **4 weeks** |
| RV100343 | $1.89\pm0.19^{c*}$ | $3.65\pm0.00^{b*}$ | $5.88\pm0.19^{a*}$ | $1.36\pm0.02^{c}$ | $3.77\pm0.10^{b*}$ | $4.85\pm0.14^{a*}$ |
| RV100199 | $1.26\pm0.05^{d}$ | $2.00\pm0.17^{c}$ | $4.57\pm0.14^{a}$ | $1.45\pm0.00^{d}$ | $2.24\pm0.12^{c}$ | $3.28\pm0.19^{b}$ |
| RV100201 | $1.62\pm0.13^{c}$ | $2.72\pm0.11^{a}$ | $2.24\pm0.07^{b}$ | $1.27\pm0.13^{c}$ | $2.59\pm0.18^{a}$ | $2.05\pm0.12^{b}$ |
| RV100332 | $1.10\pm0.14^{d}$ | $2.36\pm0.21^{c}$ | $3.09\pm0.17^{b}$ | $1.45\pm0.14^{d}$ | $2.53\pm0.19^{c}$ | $4.95\pm0.22^{a*}$ |
| RV100271 | $1.69\pm0.09^{d}$ | $2.68\pm0.18^{c}$ | $4.65\pm0.21^{a}$ | $1.11\pm0.18^{d}$ | $2.28\pm0.03^{c}$ | $3.23\pm0.15^{b}$ |
| RV100445 | $1.46\pm0.18^{c}$ | $1.90\pm0.15^{c}$ | $2.15\pm0.15^{b}$ | $1.48\pm0.19^{c}$ | $2.07\pm0.05^{bc}$ | $2.82\pm0.15^{a}$ |
| RV100333 | $1.36\pm0.16^{c}$ | $1.96\pm0.03^{c}$ | $2.03\pm0.05^{b}$ | $1.71\pm0.06^{c}$ | $2.18\pm0.18^{b}$ | $2.72\pm0.22^{a}$ |
| RV100259 | $1.41\pm0.09^{c}$ | $2.64\pm0.10^{b}$ | $2.66\pm0.19^{b}$ | $1.46\pm0.07^{c}$ | $2.39\pm0.21^{b}$ | $3.10\pm0.24^{a}$ |
| RV100265 | $1.46\pm0.09^{c}$ | $2.44\pm0.09^{b}$ | $4.10\pm0.20^{a}$ | $1.57\pm0.11^{c}$ | $2.23\pm0.12^{b}$ | $2.61\pm0.05^{b}$ |
| RV100273 | $1.44\pm0.20^{c}$ | $2.01\pm0.16^{b}$ | $2.97\pm0.21^{a}$ | $1.39\pm0.11^{c}$ | $2.05\pm0.22^{b}$ | $2.84\pm0.20^{a}$ |
| RV100511 | $1.70\pm0.12^{c}$ | $2.13\pm0.24^{bc}$ | $4.94\pm0.19^{a*}$ | $1.44\pm0.16^{c}$ | $2.66\pm0.21^{b}$ | $2.78\pm0.14^{b}$ |
| RV100432 | $1.51\pm0.04^{c}$ | $2.71\pm0.15^{b}$ | $2.82\pm0.19^{b}$ | $1.48\pm0.04^{c}$ | $3.98\pm0.18^{a*}$ | $3.44\pm0.24^{a}$ |
| RV100246 | $1.69\pm0.12^{c}$ | $2.06\pm0.07^{bc}$ | $2.98\pm0.10^{a}$ | $1.62\pm0.11^{c}$ | $2.31\pm0.02^{bc}$ | $2.82\pm0.05^{a}$ |
| RV100328 | $1.65\pm0.10^{b}$ | $2.54\pm0.18^{a}$ | $2.75\pm0.05^{a}$ | $1.62\pm0.06^{b}$ | $2.74\pm0.11^{a}$ | $2.99\pm0.19^{a}$ |
| RV100327 | $1.55\pm0.16^{c}$ | $2.59\pm0.17^{b}$ | $6.84\pm0.18^{a*}$ | $1.42\pm0.16^{c}$ | $2.40\pm0.17^{b}$ | $5.38\pm0.16^{b*}$ |
| RV100342 | $1.69\pm0.16^{c}$ | $2.18\pm0.04^{b}$ | $2.87\pm0.05^{b}$ | $2.11\pm0.13^{b*}$ | $3.40\pm0.23^{a}$ | $3.24\pm0.17^{a}$ |
| RV100330 | $1.71\pm0.13^{d}$ | $2.45\pm0.13^{c}$ | $4.23\pm0.13^{a}$ | $1.74\pm0.13^{d}$ | $2.51\pm0.20^{c}$ | $3.05\pm0.18^{b}$ |
| GBK50591 | $1.81\pm0.10^{c*}$ | $3.69\pm0.15^{a*}$ | $2.89\pm0.19^{b}$ | $2.02\pm0.02^{c*}$ | $1.99\pm0.14^{c}$ | $2.49\pm0.14^{b}$ |
| RV100438 | $1.63\pm0.07^{d}$ | $2.32\pm0.01^{c}$ | $5.66\pm0.12^{a*}$ | $1.67\pm0.03^{d}$ | $3.26\pm0.18^{b}$ | $5.73\pm0.26^{a*}$ |

Mean ± SD fresh weight (n=3).
Values on the same row with different superscripts are significantly different ($p < 0.05$) whereas means with asterisk (*) within the column were significantly higher ($P < 0.05$)

The chlorophyll contents of the leaves were shown to be higher than those of the carotenoids (Table 4.3). The chlorophylls were reported to increase with the growth of the plant. The highest chlorophyll contents were observed in mature leaves. The accessions RV100343, RV100438, RV100327, RV100332 and RV100330 reported significantly ($p < 0.05$) higher contents as compared to the others. From the results, a significant difference ($p < 0.05$) was seen for the control and stressed treatments. The stressed treatments were observed to reduce the level of chlorophylls

Table 4.3: The total chlorophyll concentration (mg/100g) in the leaves of African eggplant subjected to drought stress at different growth stages

| **Accessions** | **Stress** | | | **Control** | | |
|---|---|---|---|---|---|---|
| | **0 weeks** | **2 weeks** | **4 weeks** | **0 weeks** | **2 weeks** | **4 weeks** |
| RV100343 | $30.91\pm0.28^{b}$ | $52.01\pm2.14^{ab*}$ | $63.25\pm0.76^{a*}$ | $31.61\pm2.71^{b}$ | $59.90\pm2.50^{a*}$ | $69.74\pm1.92^{a*}$ |
| RV100199 | $19.72\pm1.36^{c}$ | $32.44\pm2.27^{b}$ | $42.27\pm3.23^{a}$ | $16.54\pm3.20^{c}$ | $43.24\pm0.64^{a}$ | $46.28\pm1.64^{a}$ |
| RV100201 | $30.18\pm3.20^{c}$ | $35.60\pm1.33^{bc}$ | $43.08\pm1.20^{b}$ | $28.72\pm1.05^{c}$ | $38.00\pm2.18^{b}$ | $47.91\pm1.26^{a}$ |
| RV100332 | $20.42\pm1.88^{c}$ | $45.13\pm1.75^{bc*}$ | $50.16\pm2.44^{b}$ | $28.49\pm1.32^{c}$ | $52.39\pm2.32^{b}$ | $63.18\pm2.20^{a*}$ |
| RV100271 | $22.25\pm1.41^{c}$ | $40.88\pm2.48^{b}$ | $44.05\pm3.26^{b}$ | $21.71\pm2.92^{c}$ | $44.06\pm0.21^{b}$ | $55.73\pm1.49^{a}$ |
| RV100445 | $30.12\pm2.87^{b}$ | $30.16\pm0.19^{b}$ | $34.42\pm2.37^{b}$ | $30.39\pm0.60^{b}$ | $41.15\pm0.91^{a}$ | $45.34\pm1.31^{a}$ |
| RV100333 | $30.03\pm0.44^{c}$ | $32.75\pm2.29^{b}$ | $38.66\pm2.47^{b}$ | $31.08\pm2.69^{bc}$ | $35.21\pm2.24^{b}$ | $46.85\pm1.80^{a}$ |
| RV100259 | $32.13\pm0.31^{c}$ | $37.91\pm1.72^{b}$ | $42.60\pm1.41^{b}$ | $29.99\pm2.58^{c}$ | $40.36\pm3.06^{b}$ | $53.87\pm1.23^{a}$ |
| RV100265 | $32.10\pm2.78^{c}$ | $37.04\pm3.67^{c}$ | $44.68\pm3.38^{b}$ | $33.68\pm1.27^{c*}$ | $39.64\pm1.29^{bc}$ | $51.04\pm2.85^{a}$ |
| RV100273 | $30.39\pm2.08^{c}$ | $31.25\pm0.85^{c}$ | $45.89\pm2.16^{ab}$ | $30.57\pm2.78^{c}$ | $37.92\pm1.31^{b}$ | $50.89\pm1.13^{a}$ |
| RV100511 | $34.35\pm2.48^{d}$ | $43.31\pm3.09^{c}$ | $46.76\pm1.32^{c}$ | $29.18\pm3.90^{d}$ | $51.40\pm0.79^{b}$ | $60.26\pm1.29^{a*}$ |
| RV100432 | $32.18\pm0.44^{c}$ | $49.15\pm1.20^{b*}$ | $45.92\pm2.20^{b}$ | $25.17\pm3.23^{c}$ | $58.13\pm2.89^{a*}$ | $54.84\pm3.61^{a}$ |
| RV100246 | $24.73\pm2.02^{c}$ | $35.96\pm0.60^{b}$ | $48.32\pm1.82^{a}$ | $32.46\pm1.36^{b}$ | $38.17\pm2.76^{b}$ | $47.35\pm3.17^{a}$ |
| RV100328 | $35.37\pm1.01^{b*}$ | $43.95\pm0.24^{a}$ | $46.93\pm2.13^{a}$ | $33.98\pm1.61^{b*}$ | $44.74\pm2.03^{a}$ | $48.34\pm2.01^{a}$ |
| RV100327 | $30.23\pm2.47^{c}$ | $34.93\pm2.74^{c}$ | $52.61\pm3.02^{b}$ | $30.14\pm2.06^{c}$ | $49.65\pm1.22^{b}$ | $66.55\pm2.55^{a*}$ |
| RV100342 | $40.46\pm1.74^{b*}$ | $46.39\pm2.11^{b}$ | $53.63\pm2.74^{a}$ | $37.01\pm2.98^{c*}$ | $53.26\pm3.41^{a*}$ | $58.31\pm1.82^{a}$ |
| RV100330 | $36.48\pm2.30^{c*}$ | $43.85\pm0.42^{b}$ | $54.49\pm1.85^{a}$ | $34.82\pm1.60^{c*}$ | $46.56\pm1.18^{b}$ | $59.99\pm1.60^{a*}$ |
| GBK50591 | $33.28\pm0.88^{c}$ | $37.56\pm3.30^{b}$ | $38.13\pm2.36^{b}$ | $29.79\pm2.61^{c}$ | $39.09\pm2.77^{b}$ | $48.16\pm1.04^{a}$ |
| RV100438 | $32.10\pm0.25^{d}$ | $41.89\pm2.13^{c}$ | $50.34\pm1.81^{b}$ | $31.32\pm2.22^{d}$ | $51.40\pm2.10^{b}$ | $62.58\pm1.20^{a*}$ |

Mean ± SD fresh weight (n=3).
Values on the same row with different superscripts are significantly different ($p < 0.05$) whereas means with asterisk (*) within the column were significantly higher ($P < 0.05$)

The identified compounds were grouped into chlorophylls (chlorophyll a and chlorophyll b) (Figure 4.2), carotenes (alpha carotene and beta carotene) (Figure 4.3) and xanthophylls (lutein, zeaxanthin, violaxanthin and neoxanthin) (Figure 4.4). In the leaf tissues, the total chlorophyll content increased with the age of the plants with mature leaves (4 weeks) reporting higher

concentration. Similarly, drought stress had significant effect on the chlorophyll content, where it was shown to reduce the total chlorophyll and chlorophyll 'a' and 'b' content in the leaves of all the accessions (Figure 4.2). Due to this, a significantly higher ($p < 0.05$) concentration of chlorophylls was therefore reported in the controls as compared to the drought stressed crops.

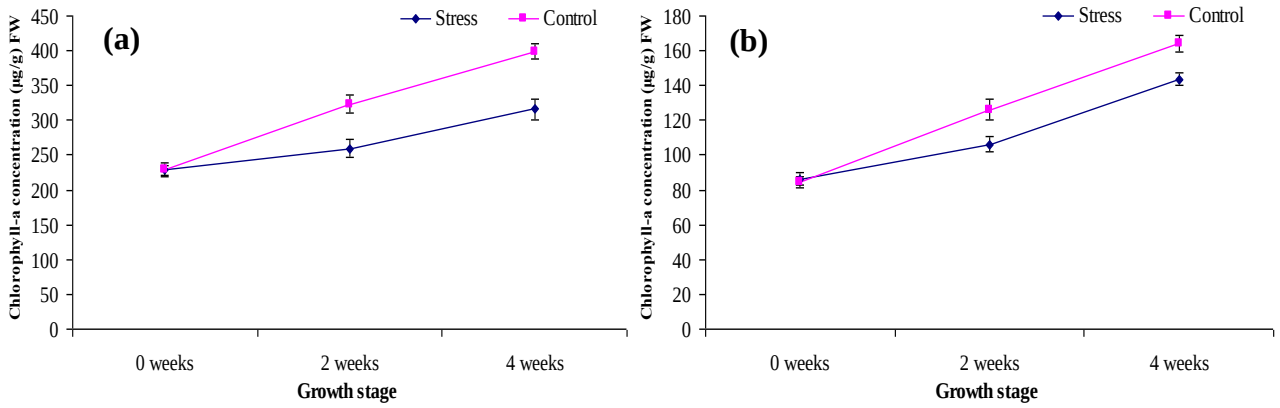



Figure 4.2: Average concentration (µg/g) of chlorophylls (a) chlorophyll a and (b) chlorophyll b in fresh weight of the drought stressed and control treatments of African eggplant leaves sampled before stress (0 weeks), 2 and 4 weeks after stress. Values are presented as mean ± SD (n=19).

The concentration of carotenes; β-carotene and α-carotene are reported in Figure 4.3. Similar to the chlorophylls, a significant increase ($p < 0.05$) in the concentration was also seen as the plant grows. Similarly to the trend in chlorophyll levels, stressed crops had significantly lower ($p < 0.05$) concentration of carotene as compared to the controls. In addition, the β-carotene was significantly higher ($p < 0.05$) as compared to α-carotene.

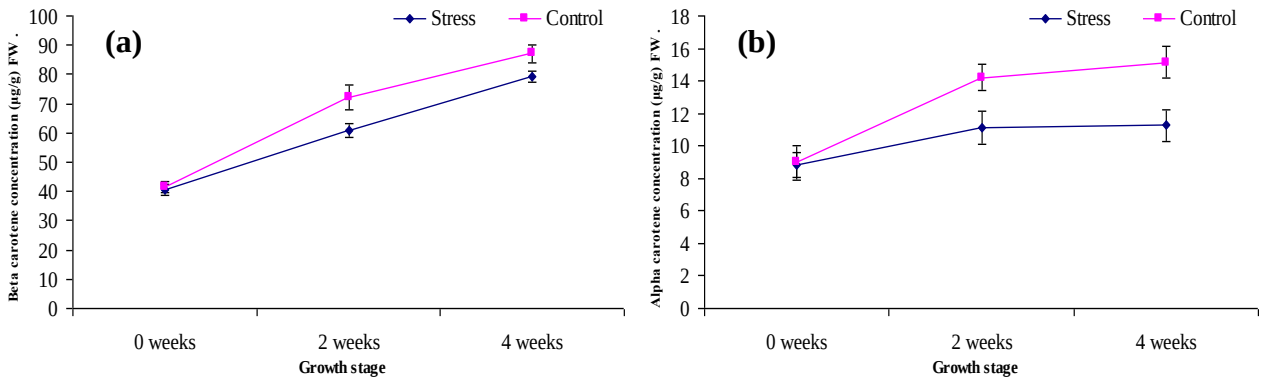



Figure 4.3: Average concentration of carotenes (a) β-carotene and (b) α-carotene (µg/g) in fresh weight of the stressed and control treatments of African eggplant leaves sampled before stress (0 weeks), 2 and 4 weeks after stress. Values are presented as mean ± SD (n=19).

Xanthophylls (oxygenated carotenoids) are an important class of target compounds, because of their antioxidant properties, their chemical stability and the difficulty of their chemical synthesis. Although ripe tomato fruits accumulated significant amounts ($p < 0.05$) of the lycopene and carotenes, only trace amounts of xanthophylls were observed. The concentration of xanthophylls lutein, zeaxanthin, violaxanthin and neoxanthin is reported in Figure 4.4. Contrary to the other xanthophylls, zeaxanthin reported increased concentration with the stress as compared to the control. Lutein had no significant difference ($p > 0.05$) between the stress and the control whereas violaxanthin and neoxanthin had significantly higher ($p < 0.05$) content in the control as compared to the stress.

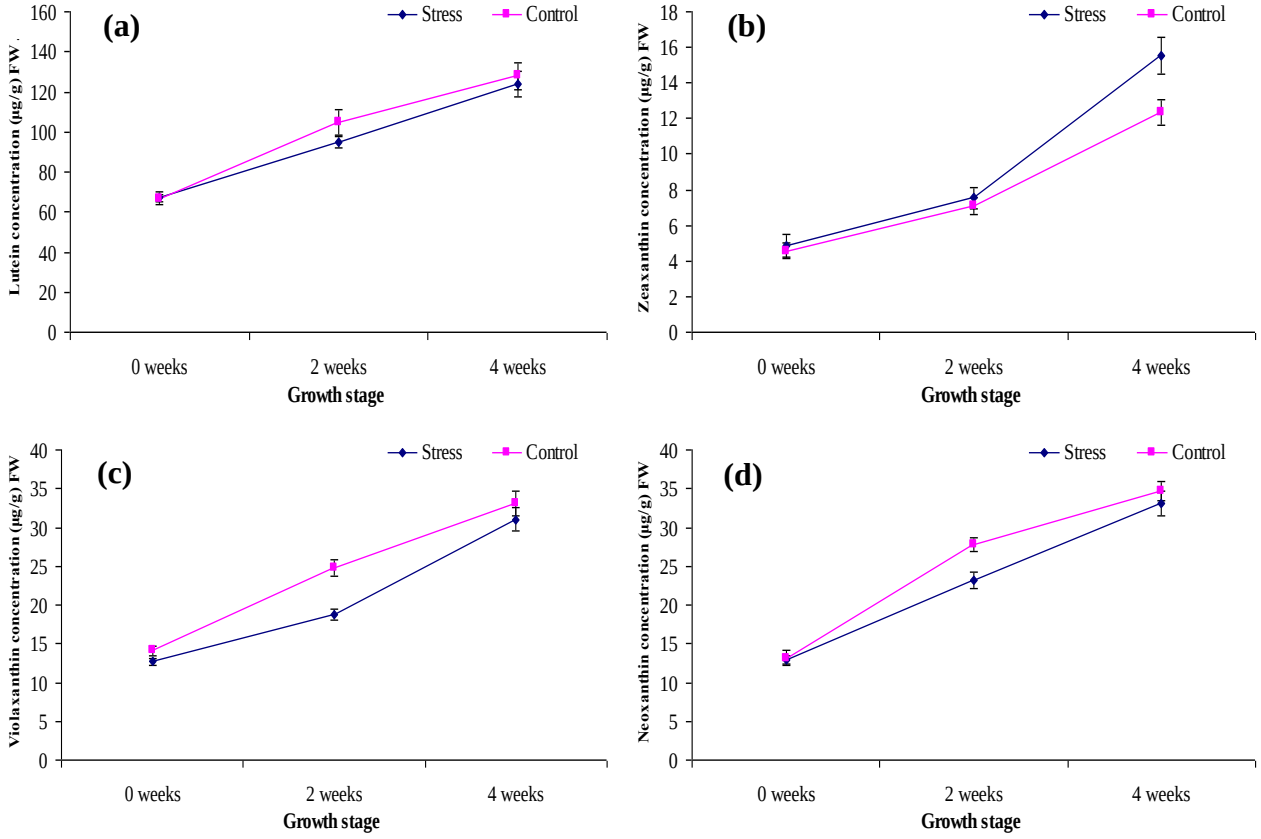



Figure 4.4: Average concentration of xanthophylls (a) lutein (b) zeaxanthin (c) violaxanthin and (d) neoxanthin (µg/g) fresh weight of the stressed and control treatments of African eggplant leaves sampled before stress (0 weeks), 2 and 4 weeks after stress. Values are presented as mean ± SD (n=19).

### 4.4.3 Effect of drought stress on the carotenoids levels of African eggplant fruits

The total carotenoid and chlorophyll contents of African eggplant fruit accessions analyzed are presented in Table 4.4 and Table 4.5, respectively. The results indicate that considerable amounts of carotenoids were observed in fruits at three different stages of ripening. The most noticeable characteristic during ripening were the dramatic changes in the carotenoid content of the fruits, where the total carotenoids increased. In contrast, the chlorophyll content was found to decrease progressively and gradually disappeared at ripe stage. The lycopene and β-carotene content appeared in significant amounts and varied significantly ($p < 0.05$) with the different ripening stages and in the individual tomato accessions (Table 4.6). Besides, it was found out that, the amount of carotenoids in the fruits depended on the accession, stage of fruit ripening and the drought stress. Major changes in fruit colour were observed during the ripening of the fruits from green to orange, colorless to red or yellow (Plate 4.2) and this explains the noticeable characteristic variation in the pigments during ripening. Additionally, it was observed that the individual carotenoid content was dependent on accession and the fruit colour with yellow fruited accessions (V1050580 and V1050589) showing high amount of beta carotene and trace amounts of lycopene. The total carotenoid contents in the fruits typically varied from 1.07±0.16 - 23.21±4.61 mg/100g fw in all treatment conditions and stage of ripening. There was a significant difference ($p < 0.05$) between the red, orange and yellow fruits. Generally, the red fruits (RV100343, RV100327, RV100246, RV100432 and RV100265) had high amount of total carotenoids as compared to the orange (RV100330) and yellow fruits (GBK50591) (Plate 4.2). However, some accessions such as RV100246, RV100265 and RV100327 had colourless fruits during the breaker stage and reported low levels of lycopene and beta carotene as well as chlorophylls. Drought stress was also seen to cause a significant increase ($p < 0.05$) in total carotenoids (Table 4.4) and reduction in chlorophylls (Table 4.5).

Table 4.4: The total carotenoid concentration (mg/100g) of the fruits of African eggplant subjected to drought stress at different ripening stages

| | Control | | | Stress | | |
|---|---|---|---|---|---|---|
| **Accession** | **Mature green** | **Breaker** | **Mature red** | **Mature green** | **Breaker** | **Mature red** |
| RV100343 | 4.90±1.17$^{d}$ | 5.44±0.48$^{d}$ | 17.75±1.04$^{b*}$ | 5.87±1.23$^{d*}$ | 11.85±0.11$^{c*}$ | 23.21±4.61$^{a*}$ |
| RV100201 | 3.72±0.62$^{b}$ | 5.55±0.29$^{b}$ | 11.00±0.45$^{a}$ | 4.26±0.57$^{b}$ | 5.82±0.11$^{b}$ | 13.93±1.72$^{a}$ |
| RV100332 | 4.10±1.12$^{c}$ | 6.85±1.32$^{c}$ | 11.72±0.87$^{b}$ | 5.32±0.14$^{c*}$ | 6.46±0.57$^{c}$ | 15.26±1.08$^{a}$ |
| RV100445 | 6.02±0.84$^{c*}$ | 11.73±0.93$^{b*}$ | 16.93±0.77$^{b*}$ | 4.27±0.22$^{c}$ | 12.26±0.12$^{b*}$ | 22.89±2.87$^{a*}$ |
| RV100259 | 6.03±2.47$^{c*}$ | 5.71±0.15$^{c}$ | 11.35±1.85$^{b}$ | 7.99±0.96$^{c}$ | 5.94±0.07$^{c}$ | 15.81±0.91$^{a}$ |
| RV100265 | 1.85±0.01$^{e}$ | 3.93±0.16$^{d}$ | 9.35±0.81$^{b}$ | 2.41±0.01$^{d}$ | 5.23±0.36$^{c}$ | 13.23±0.84$^{a}$ |
| RV100432 | 1.27±0.44$^{c}$ | 4.41±0.17$^{b}$ | 8.81±0.56$^{a}$ | 1.12±0.05$^{c}$ | 5.49±0.10$^{b}$ | 10.27±1.69$^{a}$ |
| RV100246 | 1.35±0.62$^{d}$ | 2.75±0.44$^{c}$ | 7.99±1.25$^{b}$ | 3.04±0.09$^{c}$ | 4.69±0.67$^{c}$ | 11.17±1.53$^{a}$ |
| RV100327 | 2.37±0.12$^{b}$ | 1.07±0.16$^{c}$ | 9.19±1.42$^{a}$ | 2.74±0.13$^{b}$ | 2.17±0.12$^{b}$ | 12.84±0.87$^{a}$ |
| RV100330 | 3.30±2.32$^{d}$ | 4.40±0.00$^{d}$ | 16.54±0.46$^{b}$ | 4.01±0.01$^{d}$ | 7.11±0.55$^{c}$ | 20.09±0.25$^{a*}$ |
| GBK50591 | 4.35±1.87$^{d}$ | 6.25±0.00$^{c}$ | 14.30±0.96$^{b}$ | 5.76±1.13$^{c*}$ | 8.70±0.04$^{c}$ | 19.68±1.07$^{a*}$ |

Mean ± SD fresh weight (n=3).
Values on the same row with different superscripts are significantly different whereas means with asterisk (*) within the column were significantly higher ($P < 0.05$)

Table 4.5: The total chlorophyll concentration (mg/100g) in the fruits of African eggplant subjected to drought stress at different ripening stages

| | Control | | | Stress | | |
|---|---|---|---|---|---|---|
| **Accession** | **Mature green** | **Breaker** | **Mature red** | **Mature green** | **Breaker** | **Mature red** |
| RV100343 | 15.15±0.83$^{a*}$ | 5.55±0.34$^{b*}$ | **0.81±0.07$^{c}$** | 9.94±0.43$^{a*}$ | 4.24±0.08$^{b*}$ | **1.14±0.33$^{c*}$** |
| RV100201 | 8.34±0.44$^{a}$ | 3.40±0.20$^{b}$ | **0.53±0.03$^{c}$** | 3.97±0.20$^{b}$ | 2.66±0.08$^{b}$ | **0.75±0.06$^{c}$** |
| RV100332 | 9.69±0.79$^{a}$ | 2.67±0.94$^{b}$ | **0.74±0.06$^{c}$** | 8.34±0.10$^{a*}$ | 2.38±0.40$^{b}$ | **0.30±0.00$^{c}$** |
| RV100445 | 7.75±0.19$^{a}$ | 3.06±0.66$^{b}$ | **0.92±0.05$^{c}$** | 5.06±0.08$^{a}$ | 2.03±0.08$^{b}$ | **1.30±0.10$^{c*}$** |
| RV100259 | 9.58±0.33$^{a}$ | 4.72±0.11$^{c}$ | **1.03±0.13$^{d}$** | 6.57±0.34$^{b}$ | 3.84±0.05$^{c}$ | **0.68±0.01$^{d}$** |
| RV100265 | 1.78±0.42$^{a}$ | 0.04±0.11$^{b}$ | Nd | 1.60±0.45$^{a}$ | nd | nd |
| RV100432 | 2.18±0.59$^{a}$ | 0.43±0.12$^{b}$ | **0.09±0.04$^{c}$** | 1.89±0.19$^{a}$ | nd | nd |
| RV100246 | 1.97±0.44$^{a}$ | 0.64±0.31$^{b}$ | Nd | 1.48±0.32$^{a}$ | 0.38±0.05$^{b}$ | nd |
| RV100327 | 1.95±0.52$^{a}$ | 0.39±0.11$^{b}$ | **0.04±0.01$^{c}$** | 1.19±0.47$^{a}$ | 0.22±0.08$^{b}$ | nd |
| RV100330 | 9.15±0.45$^{a}$ | 5.46±0.20$^{b*}$ | **2.19±0.03$^{c*}$** | 6.98±0.19$^{b}$ | 5.42±0.39$^{b*}$ | **1.12±0.01$^{c*}$** |
| GBK50591 | 8.34±1.02$^{a}$ | 1.29±0.16$^{b}$ | **1.01±0.01$^{c}$** | 7.27±0.40$^{a}$ | 1.73±0.30$^{b}$ | **0.96±0.00$^{c}$** |

Mean ± SD fresh weight (n=3).
Values on the same row with different superscripts are significantly different whereas means with asterisk (*) within the column were significantly higher ($P < 0.05$)
The highlighted (**bold**) mean values were significantly low and presented as (µg/100g)
nd – not detected.

Table 4.6: Average carotenoids and chlorophylls (mg/100g) of African eggplant fruits subjected to drought stress at different ripening stages

| | **Control** | | | **Stress** | | |
|---|---|---|---|---|---|---|
| | **Mature green** | **Breaker** | **Mature red** | **Mature green** | **Breaker** | **Mature red** |
| Neoxanthin | 0.67±0.20$^{a}$ | 0.46±0.14$^{b}$ | 0.25±0.04$^{d}$ | 0.58±0.14$^{a}$ | 0.42±0.13$^{b}$ | 0.34±0.07$^{c}$ |
| Violaxanthin | 0.32±0.06$^{b}$ | 0.27±0.05$^{c}$ | 0.38±0.06$^{a}$ | 0.32±0.06$^{b}$ | 0.28±0.07$^{bc}$ | 0.44±0.09$^{a}$ |
| Chlorophyll b | 1.20±0.23$^{a}$ | 0.48±0.12$^{c}$ | **0.44±0.00$^{d}$** | 0.92±0.19$^{b}$ | 0.59±0.16$^{c}$ | **0.45±0.00$^{d}$** |
| Lutein | 1.18±0.18$^{c}$ | 1.69±0.37$^{b*}$ | 0.85±0.15$^{c}$ | 1.45±0.27$^{b}$ | 2.35±0.58$^{a*}$ | 1.05±0.12$^{c}$ |
| Chlorophyll a | 5.70±1.10$^{a*}$ | 2.01±0.56$^{c*}$ | **0.40±0.00$^{d}$** | 4.01±0.77$^{b*}$ | 1.58±0.43$^{c}$ | **0.56±0.00$^{d}$** |
| Phytoene | 0.14±0.02$^{c}$ | 0.46±0.10$^{b}$ | 0.51±0.08$^{b}$ | 0.12±0.02$^{c}$ | 0.57±0.10$^{b}$ | 0.95±0.14$^{a}$ |
| Alpha carotene | 0.17±0.03$^{d}$ | 0.43±0.08$^{c}$ | 1.52±0.34$^{b}$ | 0.21±0.03$^{d}$ | 0.57±0.09$^{c}$ | 2.06±0.37$^{a}$ |
| Beta carotene | 1.08±0.15$^{d}$ | 1.96±0.38$^{cd}$ | 5.79±0.97$^{b*}$ | 1.58±0.20$^{d}$ | 2.65±0.37$^{c*}$ | 7.26±1.23$^{a*}$ |
| Cis-Lycophene | **0.21±0.00$^{c}$** | **2.14±0.00$^{c}$** | 0.72±0.12$^{b}$ | **0.28±0.00$^{c}$** | **3.41±0.00$^{c}$** | 1.15±0.19$^{a}$ |
| Trans-Lycopene | **0.17±0.00$^{d}$** | 0.02±0.01$^{c}$ | 2.90±0.64$^{b}$ | **0.23±0.00$^{d}$** | 0.03±0.01$^{c}$ | 3.86±0.80$^{a}$ |

Mean ± SD fresh weight (n=3).
Values on the same row with different superscripts are significantly different whereas means with asterisk (*) within the column were significantly higher ($P < 0.05$)
The highlighted (**bold**) mean values were significantly low and presented as (µg/100g)
nd – not detected.

Chlorophyll concentration was low in the African eggplant fruits compared to the leaf tissues (Figure 4.5). Only the mature green stage of fruit reported significantly higher ($p < 0.05$) concentration of chlorophylls. The concentration decreased gradually with the ripening of the fruit and low levels was observed in the mature red stages. The following accessions RV100265, RV100246, RV100432 and RV100327 reported low level of chlorophylls in the breaker stages and diminished levels in mature red stages. In addition, significant differences ($p < 0.05$) in the chlorophyll levels were observed between the drought stressed and control accession. Drought stressed accessions had significantly reduced ($p < 0.05$) chlorophyll levels in all the stages of ripening of the African eggplant fruits.

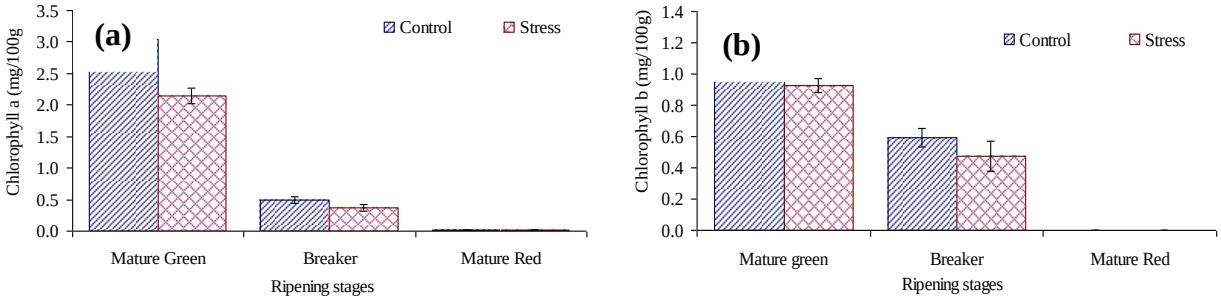



Figure 4.5: Average concentration (mg/100g) of chlorophylls (a) chlorophyll a and (b) chlorophyll b of the stressed and control treatments of African eggplant fruits during different ripening stages; mature green, breaker and mature red. Values are presented as mean ± SD (n=19).

The xanthophylls are presented in Figure 4.6 and there were no difference in their concentration between the stress and the control treatments suggesting that their degradation was not affected by drought stress. The highest xanthophyll levels neoxanthin, violaxanthin, lutein and phytoene were observed in RV100445 (2.08±0.03 mg/100g fw), RV100343 (1.08±0.02 mg/100g fw), RV100343 (7.53±0.20 mg/100g fw) and RV100445 (1.96±0.02 mg/100g fw), respectively.

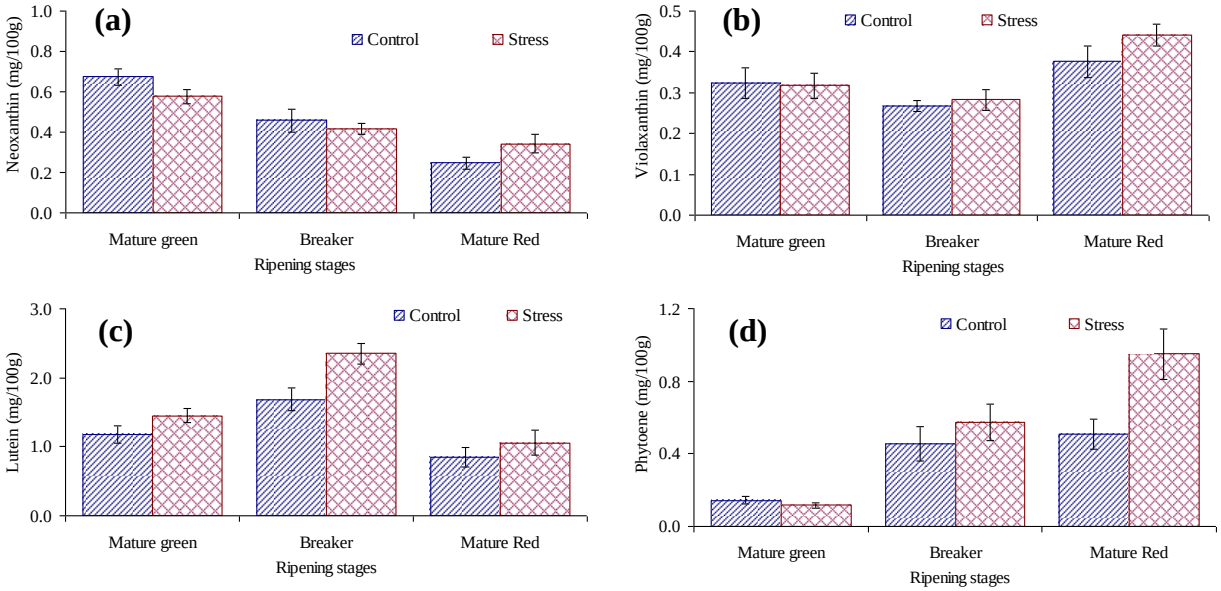



Figure 4.6: Average concentration (mg/100g) of xanthophylls (a) neoxanthin (b) violaxanthin (c) lutein and (d) phytoene of the stressed and control treatments of African eggplant fruits during different ripening stages; mature green, breaker and mature red. Values are presented as mean ± SD (n=19).

The carotene and lycopene content of the African eggplant fruit were also evaluated and average concentrations reported in Figure 4.7. The results reported lower lycopene contents as compared to the carotene content (Table 4.6). All the accessions evaluated in the present study had higher β-carotene contents in the mature stage. In addition, significant difference ($p < 0.05$) in the beta carotene level was observed between the control and drought stressed treatments and higher levels were observed in drought stressed accessions as compared to the control. This significant increase ($p < 0.05$) in β-carotene concentration during drought stress therefore indicated that β-carotene synthesis is enhanced by drought stress treatment.

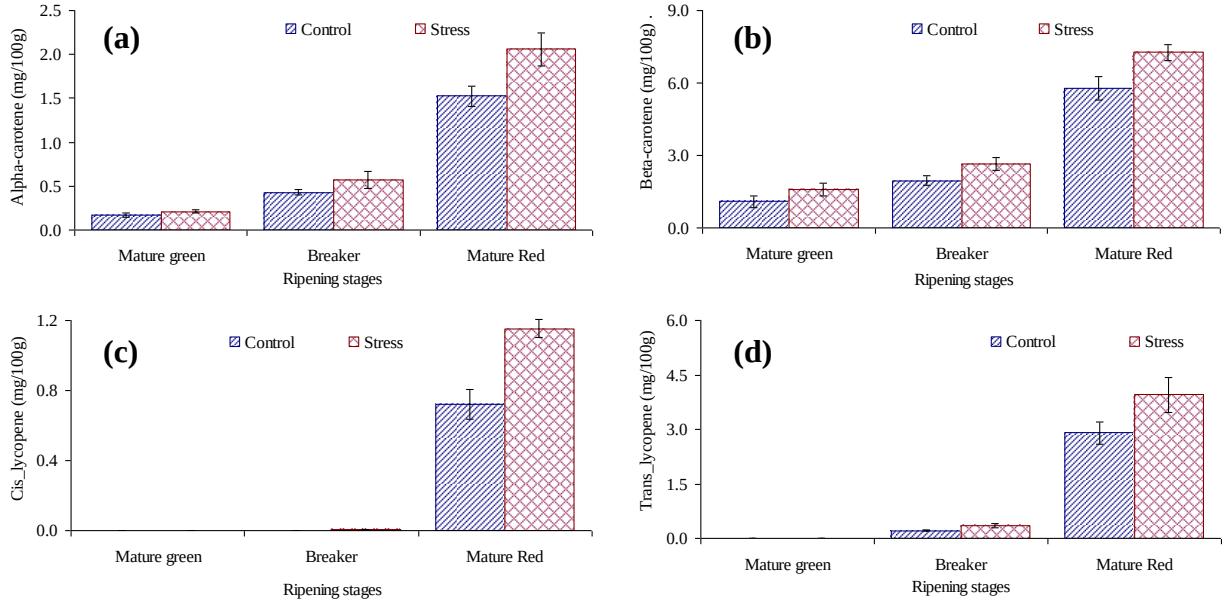



Figure 4.7: Average concentration (mg/100g) of carotenes (a) α - carotene (b) β - carotene (c) cis - lycopene (d) trans - lycopene of the stressed and control treatments of African eggplant fruits during different ripening stages; mature green, breaker and mature red. Values are presented as mean ± SD (n=19).

### 4.4.4 Effect of drought stress on carotenoid contents of African tomato fruits

African tomato accession fruits at three different ripening stages were analyzed for their pigments; carotenoid and chlorophyll contents and results are presented in Table 4.7 and 4.8, respectively. From the results, the total carotenoids in tomato fruits depended on the accession, ripening stage and the drought stress (Table 4.7). During the development of the tomato fruit from mature green to mature red or mature yellow, major changes in pigmentation were observed (Plate 4.3). The most noticeable characteristic of ripening was the dramatic change in

fruit colour from green to yellow to orange to pink to red or yellow. The total carotenoid content typically varied from 1.89±0.88 - 108.14±1.90 mg/100g fw of fruit tissue. The total chlorophylls were also observed to decrease with ripening as well as drought (Table 4.8). The total chlorophylls ranged from 0.69±0.01 µg/100g - 9.12±1.08 mg/100g for the control but for the drought stress, it was slightly lower ranging from 1.87±0.04 µg/100g - 6.33±0.50 mg/100g. Interestingly, two accessions V1050580 and V1050589 reported low chlorophyll levels at mature red stage while the others had no chlorophyll.

Table 4.7: The total carotenoid concentration (mg/100g) of the fruits of African tomato subjected to drought stress at different ripening stages

| | **Control** | | | **Stress** | | |
|---|---|---|---|---|---|---|
| **Accession** | **Mature green** | **Breaker** | **Mature red** | **Mature green** | **Breaker** | **Mature red** |
| V1005987 | 5.73±0.46$^{c*}$ | 7.54±1.31$^{c}$ | 47.11±2.01$^{b}$ | 6.37±1.59$^{c*}$ | 9.95±0.54$^{c}$ | 58.12±3.52$^{a}$ |
| V1006833 | 3.49±0.14$^{d}$ | 7.57±1.00$^{c}$ | 47.87±3.71$^{b}$ | 4.08±0.45$^{d}$ | 7.28±0.41$^{c}$ | 70.81±4.74$^{a}$ |
| V1005872 | 3.07±0.15$^{c}$ | 13.07±0.80$^{b*}$ | 72.63±1.74$^{a*}$ | 4.54±0.92$^{c}$ | 14.28±0.63$^{b*}$ | 87.85±2.93$^{a}$ |
| VI005878 | 4.51±0.65$^{c}$ | 7.94±0.37$^{b}$ | 50.55±2.38$^{a}$ | 8.36±0.40$^{b*}$ | 9.66±0.80$^{b}$ | 57.54±1.78$^{a}$ |
| V1002114 | 4.57±0.37$^{c}$ | 14.39±0.37$^{b*}$ | 101.46±2.89$^{a*}$ | 4.83±0.65$^{c}$ | 15.14±2.58$^{b*}$ | 108.14±1.90$^{a*}$ |
| V1007108 | 4.12±0.28$^{c}$ | 7.37±0.16$^{c}$ | 75.72±1.88$^{b*}$ | 5.23±0.80$^{c}$ | 9.18±0.30$^{c}$ | 100.35±3.85$^{a*}$ |
| V1050580 | 4.73±0.16$^{c}$ | 13.64±0.19$^{b*}$ | 19.84±1.13$^{a}$ | 5.28±1.54$^{c}$ | 17.15±0.68$^{ab*}$ | 22.00±1.28$^{a}$ |
| V1002112 | 2.23±0.64$^{d}$ | 5.66±0.14$^{c}$ | 62.52±1.39$^{b}$ | 2.96±0.85$^{d}$ | 6.22±0.61$^{c}$ | 81.54±3.22$^{a}$ |
| V1050589 | 5.44±0.75$^{c*}$ | 12.66±0.14$^{b*}$ | 23.12±1.31$^{a}$ | 5.71±2.10$^{c}$ | 19.00±2.51$^{a*}$ | 24.84±1.05$^{a}$ |
| V1006838 | 3.92±0.57$^{c}$ | 8.42±0.17$^{b}$ | 79.50±2.09$^{a*}$ | 3.34±0.82$^{c}$ | 9.09±0.56$^{b}$ | 96.12±1.93$^{a*}$ |
| V1006842 | 3.76±0.31$^{c}$ | 5.87±0.33$^{c}$ | 56.73±1.02$^{b}$ | 5.46±0.32$^{c}$ | 6.94±0.92$^{c}$ | 82.10±2.44$^{a}$ |
| V1006826 | 2.85±0.79$^{c}$ | 6.09±0.33$^{bc}$ | 39.78±3.34$^{a}$ | 3.00±0.60$^{c}$ | 9.16±1.79$^{b}$ | 45.56±1.21$^{a}$ |
| V1006828 | 3.08±0.45$^{d}$ | 8.20±0.18$^{c}$ | 73.88±2.14$^{b*}$ | 3.54±0.62$^{d}$ | 9.21±0.42$^{c}$ | 102.00±3.92$^{a*}$ |
| V1005874 | 2.81±0.62$^{d}$ | 8.87±0.12$^{c}$ | 59.70±1.16$^{b}$ | 3.08±0.59$^{d}$ | 10.80±0.74$^{c}$ | 71.67±2.20$^{a}$ |
| V1030380 | 2.15±0.42$^{c}$ | 5.29±0.38$^{b}$ | 60.47±3.90$^{a}$ | 2.05±1.64$^{c}$ | 6.73±0.52$^{b}$ | 72.79±1.27$^{a}$ |
| V1006892 | 4.23±0.40$^{c}$ | 8.29±0.46$^{c}$ | 58.81±3.07$^{b}$ | 5.10±1.05$^{c}$ | 8.92±0.47$^{c}$ | 76.03±2.39$^{a}$ |
| V1035028 | 2.59±0.45$^{d}$ | 5.54±0.25$^{c}$ | 41.35±2.05$^{b}$ | 1.89±0.88$^{d}$ | 5.38±0.78$^{c}$ | 50.75±2.26$^{a}$ |
| V1005875 | 4.46±0.16$^{c}$ | 10.80±0.43$^{b}$ | 41.13±1.62$^{a}$ | 5.25±0.44$^{c}$ | 12.62±1.60$^{b}$ | 48.04±1.66$^{a}$ |

Mean ± SD fresh weight (n=3).
Values on the same row with different superscripts are significantly different whereas means with asterisk (*) within the column were significantly higher ($P < 0.05$)

Table 4.8: The total chlorophyll concentration (mg/100g) in the fruits of African tomato subjected to drought stress at different ripening stages

| | Control | | | Stress | | |
|---|---|---|---|---|---|---|
| **Accession** | **Mature Green** | **Breaker** | **Mature Red** | **Mature Green** | **Breaker** | **Mature Red** |
| V1005987 | 7.73±0.77$^{a*}$ | 1.80±0.02$^{c*}$ | nd | 6.33±0.50$^{b*}$ | 1.37±0.75$^{c*}$ | Nd |
| V1006833 | 3.98±0.34$^{a}$ | 0.63±0.01$^{b}$ | nd | 3.27±0.13$^{a}$ | 0.38±0.05$^{b}$ | Nd |
| V1005872 | 3.88±0.02$^{a}$ | 1.18±0.05$^{c*}$ | nd | 2.49±0.46$^{b}$ | 1.13±0.18$^{c*}$ | Nd |
| VI005878 | 9.12±1.08$^{a*}$ | 0.84±0.04$^{c}$ | nd | 5.43±0.74$^{b}$ | 0.62±0.18$^{c}$ | Nd |
| V1002114 | 4.11±0.12$^{a}$ | 0.70±0.01$^{b}$ | nd | 3.53±0.67$^{a}$ | 0.41±0.14$^{b}$ | Nd |
| V1007108 | 6.82±0.05$^{a}$ | 1.12±0.02$^{c*}$ | nd | 3.57±0.13$^{b}$ | 0.68±0.01$^{d}$ | Nd |
| V1050580 | 4.29±0.08$^{a}$ | 1.21±0.02$^{b*}$ | **2.49±0.08$^{c*}$** | 3.91±0.13$^{a}$ | 0.86±0.52$^{b*}$ | **1.87±0.04$^{c}$** |
| V1002112 | 1.99±0.02$^{a}$ | 0.74±0.18$^{c}$ | nd | 1.15±0.21$^{b}$ | 0.39±0.36$^{d}$ | Nd |
| V1050589 | 5.87±0.14$^{a}$ | 0.68±0.02$^{b}$ | **3.02±0.07$^{c*}$** | 5.18±1.06$^{a*}$ | 0.58±0.00$^{b}$ | **2.68±0.07$^{c}$** |
| V1006838 | 3.94±0.02$^{a}$ | 1.24±0.02$^{c*}$ | nd | 2.40±0.38$^{b}$ | 0.85±0.12$^{d*}$ | Nd |
| V1006842 | 5.86±0.16$^{a}$ | 0.95±0.04$^{c*}$ | nd | 3.46±0.00$^{b}$ | 0.62±0.01$^{c}$ | Nd |
| V1006826 | 2.60±0.01$^{a}$ | 0.65±0.01$^{b}$ | nd | 2.19±0.98$^{b}$ | 0.41±0.15$^{c}$ | Nd |
| V1006828 | 3.84±0.09$^{a}$ | 0.71±0.07$^{c}$ | nd | 1.86±0.09$^{b}$ | 0.77±0.23$^{c}$ | Nd |
| V1005874 | 2.77±0.14$^{a}$ | 0.91±0.04$^{c*}$ | nd | 1.64±0.13$^{b}$ | 0.69±0.10$^{c}$ | Nd |
| V1030380 | 2.23±0.01$^{a}$ | 0.72±0.03$^{c}$ | nd | 1.90±0.14$^{b}$ | 0.45±0.69$^{d}$ | Nd |
| V1006892 | 5.12±0.36$^{a}$ | 1.18±0.08$^{c*}$ | nd | 4.49±0.12$^{b}$ | 0.62±0.11$^{d}$ | Nd |
| V1035028 | 1.88±0.06$^{a}$ | 0.49±0.03$^{c}$ | **0.69±0.01$^{d}$** | 1.44±0.11$^{b}$ | 0.32±0.01$^{c}$ | Nd |
| V1005875 | 6.13±0.10$^{a}$ | 0.51±0.01$^{c}$ | nd | 4.06±0.91$^{b}$ | 0.43±0.07$^{c}$ | Nd |

Mean ± SD fresh weight (n=3).
Values on the same row with different superscripts are significantly different whereas means with asterisk (*) within the column were significantly higher ($P < 0.05$)
The highlighted (**bold**) mean values were significantly low and presented as (µg/100g)
nd – not detected.

The average concentration of carotenoids and chlorophylls was also carried out and the results are presented in Table 4.9. The main carotenoids observed were β-carotene and lycopene. High β-carotene contents were observed in breaker stages of V1050580 and V1050589 accessions, and this accounted for 80.26 % and 89.29 % of the total carotenoids, respectively; whereas high lycopene was observed in V1005875 V1007108 V1006892 V1006826 V1006838 V1030380 V1002112 V1002114 V1006828 V1035028 V1005872 accessions accounting for between 90.01 - 92.41% of the total carotenoids.

Table 4.9: Average carotenoids and chlorophylls (mg/100g) of African tomato fruits subjected to drought stress at different ripening stages

| | **Control** | | | **Stress** | | |
|---|---|---|---|---|---|---|
| | **Mature green** | **Breaker** | **Mature red** | **Mature green** | **Breaker** | **Mature red** |
| Neoxanthin | 0.57±0.06$^{b}$ | 0.40±0.03$^{c}$ | 0.24±0.04$^{d}$ | 0.74±0.08$^{a}$ | 0.45±0.04$^{c}$ | 0.17±0.04$^{d}$ |
| Violaxanthin | 0.82±0.05$^{b}$ | 0.46±0.07$^{c}$ | 0.27±0.03$^{d}$ | 1.07±0.05$^{a}$ | 0.45±0.07$^{c}$ | 0.18±0.03$^{e}$ |
| Chlorophyll b | 1.66±0.18$^{a}$ | 0.47±0.05$^{c}$ | **3.94±0.40$^{e}$** | 1.06±0.12$^{b}$ | 0.27±0.03$^{d}$ | **2.93±0.26$^{e}$** |
| Lutein | 1.52±0.13$^{a}$ | 1.10±0.09$^{b}$ | 0.96±0.07$^{c}$ | 1.69±0.20$^{a}$ | 1.27±0.12$^{ab}$ | 1.10±0.12$^{c}$ |
| Chlorophyll a | 2.94±0.31$^{a*}$ | 0.82±0.06$^{c}$ | **22.91±1.49$^{e}$** | 2.05±0.23$^{b*}$ | 0.33±0.05$^{d}$ | **18.29±1.27$^{e}$** |
| Phytoene | **4.13±0.51$^{c}$** | 0.12±0.05$^{b}$ | 2.25±0.26$^{a}$ | **4.80±0.65$^{c}$** | 0.11±0.02$^{b}$ | 2.27±0.25$^{a}$ |
| Phytofluene | **5.22±0.49$^{d}$** | 0.09±0.01$^{b}$ | 1.36±0.10$^{a}$ | **5.25±0.43$^{d}$** | **43.69±3.89$^{c}$** | 1.36±0.14$^{a}$ |
| Beta carotene | 0.80±0.05$^{e}$ | 3.19±0.68$^{b*}$ | 1.93±0.61$^{d}$ | 0.85±0.08$^{e}$ | 4.03±0.91$^{a*}$ | 2.42±0.64$^{c}$ |
| Cis-Lycophene | **10.13±1.89$^{c}$** | 0.08±0.02$^{b}$ | 1.58±0.17$^{a}$ | **7.24±1.70$^{c}$** | 0.06±0.01$^{b}$ | 1.76±0.22$^{a}$ |
| Trans-Lycopene | **37.28±3.54$^{e}$** | 3.17±0.52$^{c*}$ | 46.39±4.64$^{b*}$ | 0.09±0.01$^{d}$ | 3.88±0.59$^{c*}$ | 60.32±5.93$^{a*}$ |

Mean ± SD fresh weight (n=3).
Values on the same row with different superscripts are significantly different whereas means with asterisk (*) within the column were significantly higher ($P < 0.05$)
The highlighted (**bold**) mean values were significantly low and presented as (µg/100g)
nd – not detected.

Similar to African eggplant fruits, the African tomato fruits reported the same trend in the content of chlorophylls. Regardless of accession, accumulation of chlorophylls was observed in the mature green stage of fruit and the concentration decreased with the ripening of the fruit reaching considerably low levels during mature red stages (Figure 4.8). The mature green fruits of the following accessions V1005878, V1005987, V1007108 and V1005875 reported higher chlorophyll contents whereas the accessions V1002112, V1005874, V1035028 and V005028 reported low levels. Besides, a significant variation in the chlorophyll levels were observed in the drought stressed and control plants. In this case, drought stress had a significant reduction ($p < 0.05$) effect in the levels of chlorophylls. In general, drought stress decreased the chlorophyll content in all the ripening stages.

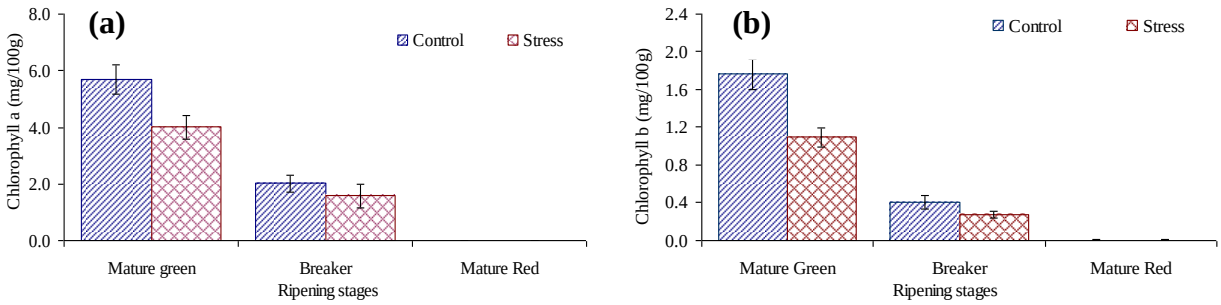



Figure 4.8: Average concentration (mg/100g) of chlorophylls (a) chlorophyll a and (b) chlorophyll b of drought stressed and control African tomato fruit during different ripening stages; mature green, breaker and mature red. Values are presented as mean ± SD (n=18).

The results of xanthophylls are reported in Figure 4.9, and the levels of individual xanthophylls were observed to be significantly low ($p < 0.05$) in the African tomato fruit accessions as compared to lycopene and carotenes. From the results (Table 4.9), drought stress did not significantly affect the level of xanthophylls ($p > 0.05$), an indication that most xanthophyll degradation was not dependent on drought stress. Although there was no significant difference ($p > 0.05$) in the phytoene and phytofluene concentration between the stress and the control treatments, the lutein level was increased slightly during stress. Violaxanthin and neoxanthin was shown to decrease with fruit ripening with mature green fruits reporting significantly higher ($p < 0.05$) levels as compared to mature red. On the other hand, phytoene and phytofluene reported a contrary result with increasing concentration.

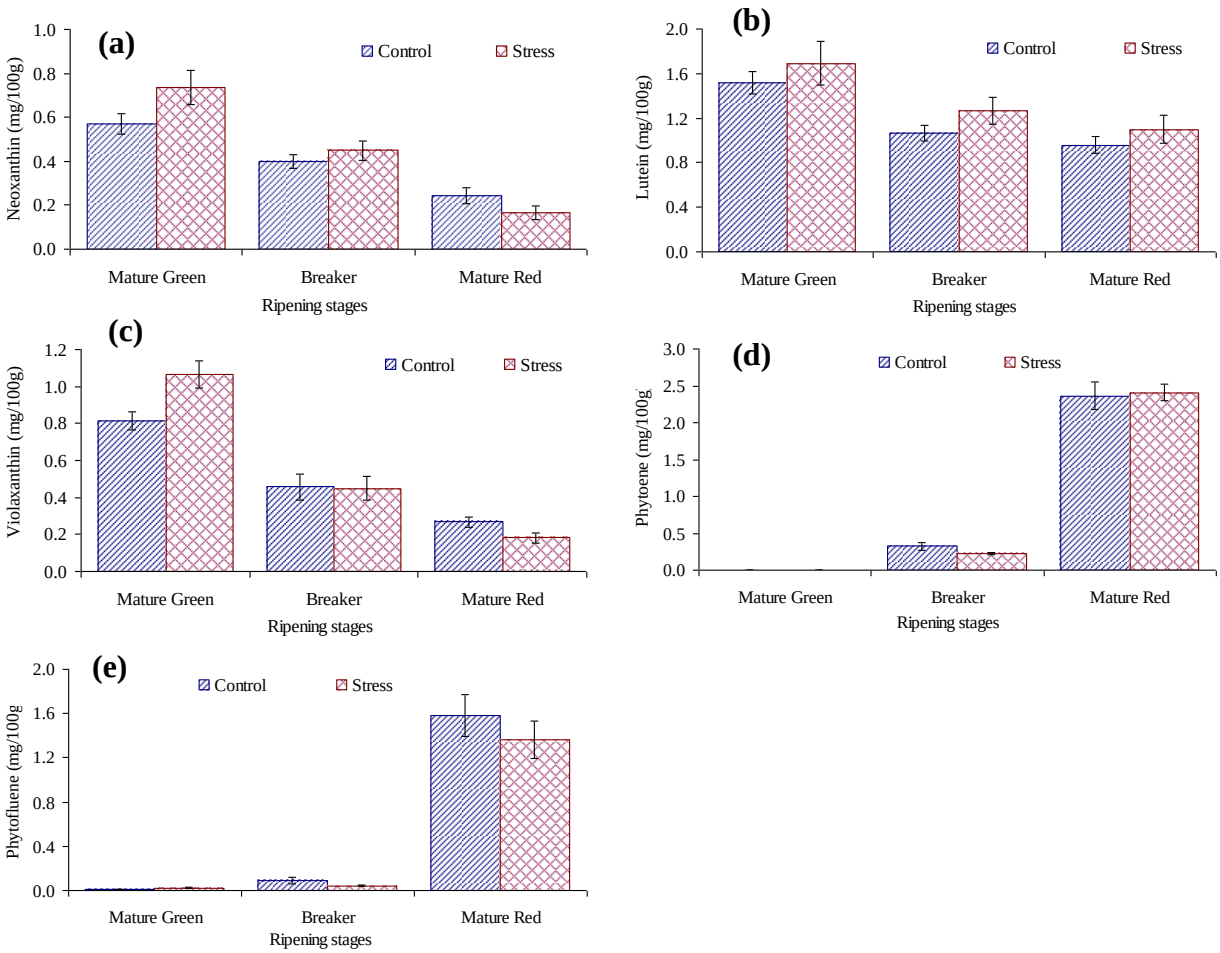



Figure 4.9: Average concentration (mg/100g) of xanthophylls (a) Neoxanthin and (b) Lutein (c) Violaxanthin (d) Phytoene (e) Phytofluene of drought stressed and control African tomato fruit during different ripening stages; mature green, breaker and mature red. Values are presented as mean ± SD (n=18).

The result of carotenes and lycopene of the African tomato fruits showed a significant difference ($p < 0.05$) between the different ripening stages (Figure 4.10). The accessions V1006826, V1035028, V1005875, V1030380 and VI005878 presented the lowest content of β-carotene 0.58±0.02 0.65±0.06 0.81±0.12 0.82±0.05 0.82±0.02 mg/100g fw, respectively, while the fruits of cherry type accessions, V1050580 and V1050589 presented significantly higher ($p < 0.05$) β-carotene content of 13.76±0.29 and 16.97±0.60 mg/100g fw, respectively. The study provides results that are consistent with the findings of previous studies which have found that cherry tomatoes are richest in β-carotene (Adalid *et al.*, 2010). In most accessions, the β- carotene was high in the breaker stage as compared to the mature red stage. In addition, there was significant

difference ($p < 0.05$) between the lycopene content of the control and drought stressed treatments. Nevertheless, the β-carotene content also changed in relation to the fruit colour with the yellow fruited accessions V1050580 and V1050589 reporting significantly high ($p < 0.05$) β-carotene than the red-fruited accessions. The significant increase ($p < 0.05$) in β-carotene concentration therefore indicated that β-carotene synthesis is enhanced by drought stress treatment.

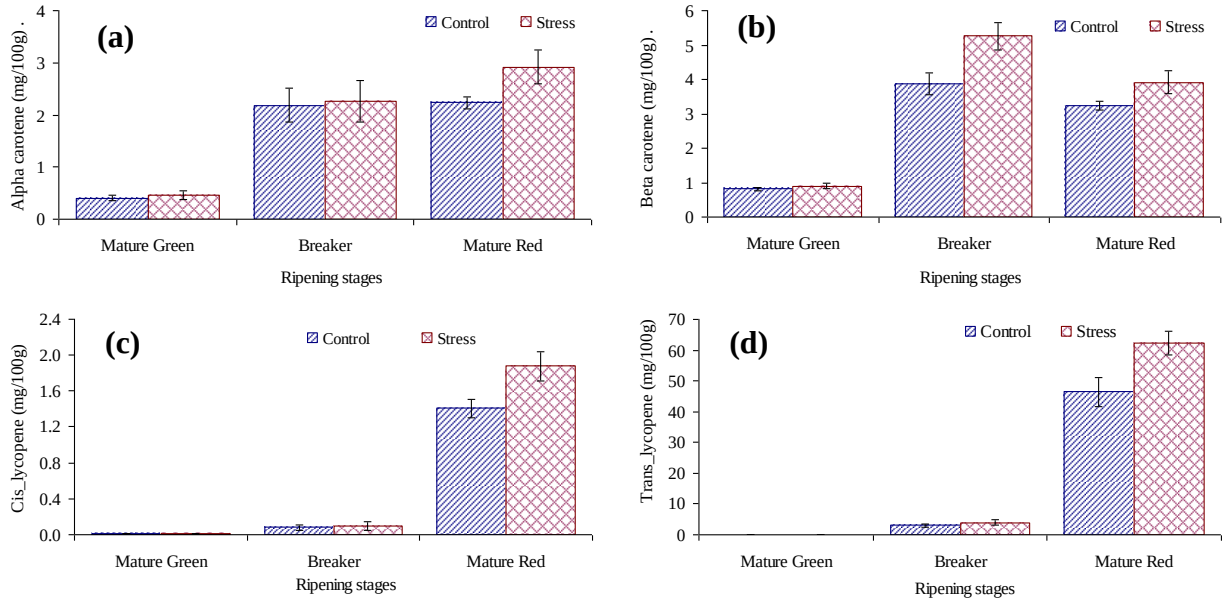



Figure 4.10: Average concentration (mg/100g) of carotenes and lycopene (a) α-carotene (b) β-carotene (c) cis-lycopene (d) trans-lycopene of African tomato fruit during different ripening stages; mature green, breaker and mature red. Values are presented as mean ± SD (n=18).

On the other hand, the study found consistent and significant variation in lycopene concentrations between the analyzed tomato accessions. It was observed that the harvest maturity affected lycopene content as observed in the three stages of ripening. Contrary to the chlorophyll content which was found to decrease progressively and gradually disappeared at ripe stage, the lycopene content increased. Although significantly low ($p < 0.05$) levels of total lycopene (µg) were observed in the mature green stage (Table 4.9), the levels considerably increased in the breaker stage and significantly ($p < 0.05$) accumulated in the final period (mature red stage) of ripening. Higher amounts were found in V1006828 and V1002114 which reported 93.47±1.81 and 99.36±0.75mg/100g, respectively. The high lycopene content in the remarkable accessions contributes to the attractive appearance of their fruits. Although the lycopene content increased with the ripening, there was significant variation ($p < 0.05$) among the individual tomato

accessions. However, depending on the individual tomato accession, the mean values of lycopene was in the range of between 7.66 - 99.36 mg/100g fw for mature red stages and 0.10 - 9.19 mg/100 fw breakers and 8.28 – 196.06 µg/100g fw for mature green fruits. Even though lycopene content is not linearly related to colour changes, the current study demonstrates an association and a positive correlation between lycopene and the red colour. Since the different selected accessions had distinct colour change from yellow to red, it was observed that the red fruited accessions recorded significantly higher ($p < 0.05$) lycopene content ($\geq$ 15 mg/100g, fw) as compared to the yellow or orange accessions ($\leq$ 10 mg/100g fw).

## 4.5 DISCUSSION

This study reported carotenoids which included neoxanthin, violaxanthin, zeaxanthin, α-carotene, β-carotene, lutein, phytoene, phytofluene and lycopene. Other photosynthetic compounds such as chlorophylls (chlorophyll a and chlorophyll b) were also identified. These compounds were found to be significantly affected by plant growth, fruit ripening stages and drought stress.

For the African eggplant leaf tissues, the concentration of the different carotenoids and chlorophylls was seen to vary between the different accessions. The different growth stages reported distinct carotenoid concentrations with the old leaves (4 weeks after stress) reporting higher concentrations as compared to the other stages. This clearly indicates that the plant pigments accumulate as the plant grows. This concurs with the findings of other studies which have reported similar results in tomato plants (Moco *et al.*, 2007; Dorais *et al.*, 2008). Contrary to this, RV100201, RV100432 and RV100342 accessions reported significantly higher ($p < 0.05$) carotenoids at 2 weeks and the contents reduced at 4 weeks. The fact that mature leaves suffered more stress than young leaves in these accessions suggests that developmental stages of these leaves might contribute to the differential prevention of oxidative damage in mature plants. The observed changes in carotenoids therefore could directly be related to known phenomenon associated with plant development, stress and photosynthetic activity (Liu *et al.*, 2015)

The results also demonstrate increased carotenoid contents during drought stress. The major carotenoids in leaves (violaxanthin and β-carotene) increased progressively in control, whereas

the proportion of lutein did not significantly change ($p > 0.05$) during the stress treatment. This could be an indication that lutein is not affected by drought stress and this is an important avenue in the study of plant tolerance to stress. Variations similar to those of chlorophyll have also been observed for lutein, β-carotene and neoxanthin in rosemary plants (Munné-Bosch and Alegre, 2000). The proportions of zeaxanthin, neoxanthin, and violaxanthin were largely altered by the drought stress treatment. During the stress treatment zeaxanthin increased whereas violaxanthin and neoxanthin decreased. Under many environmental circumstances zeaxanthin is produced from violaxanthin, and therefore its accumulation could possibly be used as an indicator of stressed tissue (Jangid and Dwivedi, 2016). This demonstrates an important factor of drought stress in enhancing carotenoid synthesis. This may provide information about the level of stress experienced by the plant as well as its ability to endure these stresses (Strzalka *et al.,* 2003). Besides, it has been observed that drought stress leads to inhibition of cell division and enlargement leading to reduction in vegetative and reproductive growth (Yordanov *et al.,* 2003; Zlatev and Lidon, 2012). Therefore, the decreased leaf area ratio reduces the photosynthesizing area and finally growth rate. Additionally, it has been observed that low level of soil water disturbs water relation in plant, which is directly related to uptake of water and mineral (Yordanov *et al.*, 2003).

In addition, the results showed that drought stress significantly affects ($p < 0.05$) the levels of chlorophylls which are the primary photosynthetic pigments. The limitation of water supply has also been shown to induce faster chlorophyll degradation in previous experiments (Kiani *et al.*, 2008; Kumar *et al.*, 2011a; Heba and Samia, 2014). This is confirmed by the findings of Farooq *et al.* (2009) which observed that both chlorophyll a and b are prone to soil drying. The values in this study were also seen to fall within the wide ranges of data found in the literature where a reduction in chlorophyll content has been reported during stress (Massacci *et al.*, 2008; Jaleel *et al.*, 2009). The decrease in chlorophyll content under drought stress may be because of reduction in activity of the enzymes involved in chlorophyll synthesis (Jangid and Dwivedi, 2016), or due to increase in chlorophyll break down caused by ROS (Kaewsuksaeng, 2011). Drought stress leads to dehydration of mesophyll cell and reduction in water use efficiency, which decreases photosynthetic rate. Moreover, drought has been reported to cause closing of stoma, limitation of gas exchange and reduction of leaf area (Jaleel *et al.,* 2009; Kumudini, 2010); consequently

decreasing photosynthetic pigments and activity. This might be attributed to reduced synthesis of the main chlorophyll pigment complexes encoded by the cab gene family (Nikolaeva *et al.*, 2010), or to destruction of the pigment protein complexes which protect the photosynthetic apparatus, or to oxidative damage of chloroplast lipids and proteins, therefore formation of chlorophyll a, b and other carotenoids decreases. Stomatal and non-stomatal limitations (metabolic impairment) are generally accepted to be the main determinant of reduced photosynthetic pigments under drought stress (Farooq *et al.*, 2009). The retardation in the content of chlorophylls in response to drought stress might be attributed to the ultra-structural deformation of plastids including the protein membranes forming the thylakoids. This is because drought stress has been reported to have the ability to reduce the tissue concentrations of chlorophylls, primarily with the production of ROS in the thylakoids (Reddy *et al.*, 2004). Due to the significant decrease ($p < 0.05$) in chlorophylls during drought stress, it is evident that drought may lead to reduction in plant productivity. This is mainly by inhibiting growth and photosynthesis, and is one major limiting factor in agriculture worldwide leading to huge reductions in crop yield (Chaves and Oliveira, 2004).

The main carotenoids detected in the African eggplant and tomato fruits were neoxanthin, violaxanthin, β-carotene, lycopene and lutein as described by Moco *et al.* (2007). A significant variation ($p < 0.05$) in carotenoid concentrations between the accessions was observed and the concentrations depended on the accessions. For the African eggplant fruits, RV100343 reported significantly higher ($p < 0.05$) concentration of carotenoids as compared to the other accessions. On the other hand, some African eggplant accessions such as RV100265, RV100432, RV100246 and RV100327 reported lower levels of carotenoids and chlorophylls. This may be attributed to the colour of the fruits since they were white in the breakers stage. For the African tomato accessions, V1006838, V1007108, V1006828 and V1002114 accessions reported the highest total carotenoid levels whereas V1050580 and V1050589 accessions had lower carotenoid contents. In addition, the plum-type accessions particularly V1006838, V1006828 and V1002114 had significantly high ($p < 0.05$) levels of lycopene as compared to the cherry-type accessions and this concur with findings by Muratore *et al.* (2005) which reported that plum-shaped tomatoes have the highest lycopene content when compared to cherry and round tomatoes. Although the cherry type accessions reported lower lycopene content, the red cherry tomato

types showed a significantly higher ($p < 0.05$) lycopene content as compared with the yellow cherry accessions. This data corresponds to earlier studies which reported lycopene content between 1.95 - 4.62 mg/100g fw (Dar and Sharma, 2011), 0.57 mg/100g- 4.78 mg/100g (Kuti and Konuru, 2005) and 8.0 – 3.7 mg/100g fw (Frenich *et al.*, 2005). Besides, the average lycopene content of raw tomatoes has been reported at 3.0 mg/100g fw (Kuti and Konuru, 2005; Adalid *et al.*, 2010).

Ripening stages were also observed to significantly affect ($p < 0.05$) the carotenoids particularly lycopene and chlorophylls. This was observed in the three stages of ripening whereby significantly low ($p < 0.05$) levels of carotenoids and high levels of chlorophylls were observed in the mature green stage. The most noticeable characteristic was the changes in the colour from green to red during ripening. The change in fruit pigmentation from green to red might have been caused by a massive accumulation of carotenoids; lycopene and β-carotene within the plastids and the degradation and disappearance of chlorophylls (Dorais *et al.*, 2008). This may be because the chloroplasts of the mature green fruit are transformed into chromoplasts, which accumulate lycopene in membrane-bound crystals (Dorais *et al.*, 2008). This concurs with the findings of other studies which have reported that lycopene and β-carotene are among the major carotenoids in tomato fruits (Fraser *et al.*, 1994; Dorais *et al.*, 2008). Based on individual accessions, it was seen that the level of carotenoids increased with fruit ripening stages with mature green reporting significantly lower ($p < 0.05$) carotenoid but higher chlorophyll levels. The total carotenoids increased in the breaker stage and in most of the accessions significant accumulation were observed in the final stage (mature red stage) of ripening and this can be attributed to the high lycopene in red fruits. Although lycopene was absent in the African eggplant leaves, significantly high ($p < 0.05$) concentration was observed in the African eggplant and tomato fruits. From the results, it was evident that lycopene was the main contributor in the total carotenoids accounting for up to 92% of the total carotenoid content. The fruits with higher lycopene content reported significantly higher ($p < 0.05$) total carotenoid content. This is because lycopene was observed to be the main carotenoid accounting for significant amount of total carotenoids in the fruits. The results of this study concur with the findings by Deák *et al.* (2015) which reported high lycopene contents accounting for from 65 - 98% of the total carotenoid content in tomato fruits, depending on the cultivar. Similarly other studies have reported that β-

carotene and lycopene are predominant carotenoids in yellow and red ripe fruits, respectively and majorly contributes to the observed increase in the total carotenoid levels at mature ripe stage of the fruits (Bramley, 2002; Brandt *et al.*, 2006; Pék *et al.*, 2010). The remainder has been reported to be composed of α-carotene and traces of lutein, zeaxanthin, lycoxanthin as well as several minor carotenoids in tomatoes.

Besides the high lycopene content in ripe fruits, it was evident that drought stress has an influence on the levels of the carotenoids. In this study, a significant variation in the levels of the major carotenoids was observed for control and drought stressed accessions, with drought causing significant increase ($p < 0.05$) in lutein, β-carotene, neoxanthin and lycopene and reduction in chlorophylls. The accumulation of carotenoids during stress therefore demonstrates their function as photoprotectants. This is because carotenoids have been shown to have the ability to neutralize harmful byproducts of photooxidation generated during stress and protect chlorophylls (Clinton, 1998) therefore important in plant stress tolerance. Various phytohormones are involved in fruit development and ripening as reported by Srivastava and Handa (2005). These phytohormones contribute to the observed quantitative and qualitative changes in the carotenoid profile (Fraser *et al.*, 2007). In addition, stress has been reported to induce upregulation of the genes encoding for the enzymes involved in the key steps of lycopene biosynthesis (Liu *et al.*, 2015) and this may explain the increased lycopene level detected in the drought stressed tomatoes. Similarly, overexpression of key enzymes involved in carotenogenesis was observed in transgenic tobacco under drought and salt stress (Cidade *et al.*, 2012). Moreover, a concerted action of both enzymatic (ascorbate peroxidase, superoxide dismutase, peroxidase, ascorbate peroxidase, catalase, polyphenol oxidase and glutathione reductase) and non-enzymatic antioxidant (ascorbate, reduced glutathione, α-tocopherol and carotenoids) systems alleviates oxidative damage generated by drought stress in the plant tissue (Prochazkova *et al.*, 2001). Carotenes form a key part of this plant antioxidant defense system, but they are very susceptible to oxidative destruction. In severe stress, β-carotene may be rapidly destroyed and therefore are no longer available to protect against oxidative damage (Young and Britton, 1990). This may explain the significant reduction in the concentrations of the stressed crops from the study.

Since lycopene was the main carotenoid, high accumulation was observed in the study during stress indicating that its synthesis is enhanced by drought stress treatment. This concurs with the findings of other studies which observed that lycopene is magnified by environmental conditions and agricultural practices, especially those affecting plant nutrient status (Abushita *et al.*, 2000; Dumas *et al.*, 2003). Similar study by Giannakoula and Ilias (2013) reported significant increase in lycopene in tomato genotypes under drought stress condition. Lycopene is synthesized from phytoene, through a series of desaturation reactions (Bramley, 2002) and it is one of the biochemical precursors and a key intermediate in the biosynthesis of other important carotenoids such as β-carotene and xanthophylls (Enfissi *et al.*, 2005). It has a specific role in defense mechanism against environmental stress by scavenging peroxyl radicals and quenching singlet oxygen (Giannakoula and Ilias, 2013). The genes mediating lycopene synthesis during fruit ripening are up-regulated and those mediating its cyclization are downregulated, resulting in the accumulation of this compound in ripe fruits (Su *et al.*, 2015). The results concur with the findings of other studies which have reported that lycopene is predominant carotenoid in red ripe tomato fruits and majorly contributes to the observed increase in the total carotenoid levels at mature ripe stage of the fruits (Bramley, 2002; Brandt *et al.*, 2006; Pék *et al.*, 2010). The significant composition of carotenoids in the African eggplants and tomatoes therefore explains their nutritional and nutraceutical importance and may lead or assist in the breeding programs of these crops. Interestingly, other studies on African tomato fruits have shown that controlled drought stress does not significantly affect yield but has a significant effect on important quality aspects such as ascorbic acid and carotenoids (Tembe *et al.*, 2017). Therefore, drought stress offers an excellent prospect for enhancing carotenoid accumulation to improve the cultivated crops. Controlled level of stress, imposed via reduced irrigation may therefore be exploited to enhance quality attributes of the tomatoes and eggplants.

## 4.6 CONCLUSION

This study gave comparative profiles of carotenoids at different growth and ripening stages under drought stress. The increased carotenoid concentration with plant maturity indicates that mature leaves (4 weeks old in this case) can provide high amounts of carotenoids that are important to human health. Similarly, the changes in fruit colour (from a green to a red) confirms significant change in carotenoids particularly lycopene and β-carotene as well as photosynthetic

pigments chlorophylls. The results also indicate the importance of ripe fruits in providing the much needed carotenoids and can be incorporated in the diet to promote human nutritional requirements and health. Since the most pronounced differences in the carotenoids were observed between control and stressed leaves and fruits, it is evident that drought stress plays a major role in enhancing lycopene and potentially other carotenoids. The leaves of the stressed crops reported significantly decreased amount of carotenes, chlorophylls, neoxanthin and violaxanthin while the concentration of zeaxanthin increased with stress and lutein had no significant change. In conclusion, this study presents information that attests to the importance of African eggplants and tomatoes in providing the much-needed dietary nutraceutical potential since they have substantial amounts of the important carotenoids. Furthermore, the accessions that performed well under drought stress; RV100343, V1006838, V1007108, V1006828 and V1002114 are good candidates for eggplant/tomato improved programs targeting nutritional quality and will definitely add value to the study of stress tolerance in crops.

# CHAPTER FIVE

# METABOLOMIC ANALYSES TO EVALUATE THE EFFECT OF DROUGHT STRESS ON SELECTED AFRICAN EGGPLANT AND TOMATO ACCESSIONS

## 5.1 ABSTRACT

African eggplants and tomato are highly valued vegetable and fruit crop for their health-promoting effects. They are known to be well adapted to various environmental stresses. Thus, understanding their response mechanisms to drought stress is of great importance. In this study, a GC-MS and LC-MS metabolomic approach was applied to evaluate the effect of drought stress on metabolites at different stages of growth and at different ripening stages. Significant changes ($p < 0.05$) in metabolite contents were observed and potentially important metabolites with respect to stress responses were characterized. Proline, glutamate, sucrose, fructose and tricarboxylic acid cycle metabolites were shown to accumulate with stress. Flavonoids particularly quercetin and kaempferol derivatives were also affected by stress. Highest positive correlations were observed between in hydroxybenzoic acid and 5-caffeoylquinic acid ($r = 0.984$), caffeic acid and 5-caffeoylquinic acid ($r = 0.969$); ferulic acid-hexose and GABA ($r = 0.972$); tri caffeoylquinic and 5-caffeoylquinic acid ($r = 0.995$); naringenin hexose and naringenin dihexose ($r = 0.978$); cinnamic acid and rutin ($r = 0.977$); tri caffeoylquinic acid and hydroxybenzoic acid ($r = 0.981$). Principal component analysis (PCA) showed a clear discrimination between the different accessions, growth stages, ripening stages and stress. The results therefore illustrate the significant effect of drought stress on the concentrations of some primary metabolites such as amino acids, sugars and organic acids and secondary metabolites such as flavonoids.

## 5.2 INTRODUCTION

Despite the African Solanaceae accessions thriving well in drought-prone areas, water stress may affect their physiological, biochemical and molecular processes such as metabolism (Boutraa, 2010; Bedon *et al.,* 2011; Azadeh *et al.,* 2014). Metabolism is the end result of the biochemical dynamics of plants starting with gene expression. Metabolomics is the identification and quantitation of all low molecular weight metabolites in a given organism, at a given

developmental stage and in a given organ, tissue or cell type (Fiehn, 2001). It an essential part of a systems biology approach to study plant defense, since different metabolic profiles are indicative of changes in metabolic pathways (López-Gresa *et al.*, 2010; Kusano *et al.*, 2011). It is a powerful approach for revealing integrated networks in plants (Fiehn, 2002) and has already helped researchers clarify the complex mechanisms of cellular metabolic pathways in response to various abiotic stresses (Bundy *et al.*, 2009). When plants are subjected to drought stress, they change physically and chemically in numerous ways by producing a huge number of metabolites or accumulation of osmolytes to adapt to the stress conditions (Urano *et al.*, 2009). Therefore, metabolic responses of plants to water deficit can be viewed as potentially adaptive changes that reflect ordered operation of metabolic regulatory mechanisms, and which favor the performance of the plants as a whole during or after stress. Understanding the relationship between the accumulation of osmolytes and stress tolerance is of great relevance to crop yield (Serraj and Sinclair, 2002). Metabolomics therefore contributes significantly to the study and understanding of stress biology in plants by identifying different compounds in response to its environment and the part they play in acclimation or tolerance response (Jorge *et al.*, 2014). The most popular metabolomics techniques focus on metabolites with similar and specific chemical properties and are globally known as metabolite profiling only covering up a fraction of the metabolome (Fernie *et al.*, 2004). To achieve a comprehensive coverage of the vast range of metabolites, several analytical techniques consisting of separation techniques have been applied. Gas chromatography mass spectrophotometer (GC-MS) and liquid chromatography mass spectrophotometer (LC-MS) has been widely used to monitor and characterize the degree of metabolic impact induced by drought stress (Roessner *et al.*, 2001; Kopka *et al.*, 2004). According to Fernie *et al.* (2004) the method allows the detection and robust quantification of vast metabolites of known chemical structure including organic acids, sugars, sugar alcohols, amino acids and a few soluble secondary metabolites. The responses of many plant species to drought stress have been extensively studied (Gupta *et al.*, 2013) and several studies have been carried out on many of the wild species. The most important abiotic stress factors, such as drought, salinity, soil flooding and extreme temperatures, cause significant changes in the composition of the plant metabolome (Obata and Fernie, 2012).

Besides, more metabolomic studies on Solanaceous species of crops has been carried out under different abiotic and biotic stresses. Comparative metabolomics to assess susceptibility of tomato to biotic stresses such as tomato yellow leaf curl virus infection (Sade *et al.*, 2015) and root-knot nematode infestation (Eloh *et al.*, 2016) revealed change in metabolite levels. In addition, most studies have reported effects of drought stress on concentration of different metabolites for example in tobacco (Rabara *et al.*, 2015), tomato (Schauer *et al.*, 2005; Semel *et al.*, 2007), potato (Vazquez-Robinet *et al.*, 2008) and eggplant (Senyigit *et al.*, 2011). On the other hand, studies within sections of the wild Solanaceae suggest that species harbor high chemical diversity (Schauer *et al.*, 2005). In spite of this, limited attention had been paid to studies covering drought stress at development stages of African Solanaceae and little data on the whole set of metabolites present during drought stress is available up to now.

Therefore, this chapter involved the application of metabolomics in determining the effect of drought stress on the metabolite composition of African eggplants and tomatoes at different developmental and ripening stages. GC-MS and LC-MS metabolomic analysis was carried out on the leaves and fruits of African eggplants and the fruits of the African tomato. From this study, metabolic adjustments in response to the drought stress conditions may highlight pools of metabolites that play important roles in metabolism and physiology of the plant during drought.

## 5.3 MATERIALS AND METHODS

The frozen leaf and fruit tissues of the selected African eggplant and tomato accessions outlined in Chapter four sections 4.3.2.1 and 4.3.2.2 were used for GC-MS and LC-MS metabolomic analysis. All the reagents used in this analysis were purchase as shown in Appendix 1.

### 5.3.1 Metabolite extraction and derivatization for GC-MS analysis

The GC-MS analysis was carried out by a method modified from Schauer *et al.* (2005) and Ruprecht *et al.* (2014). Frozen leaf tissues (100 mg) of each sample was weighed and transferred to 2 ml Eppendorf tubes. Extraction solvent; methanol: isopropanol: glacial acetic acid (80:19:1, v/v) containing ribitol (1.25 µg/ml extraction solvent) as internal quantitative standard was added in the ratio of 10 µl solvent per 1 mg sample. Two beads were placed in each tube and vortexed or homogenized with FastPrep machine (FastPrep-2 4, MP Biomedicals, Santa Ana, California,

USA) for 2 min and left to stand for 30 min. It was then centrifuged at 10,000 rpm for 15 min at RT. Four hundred microliters (400 µl) of the supernatant was evaporated using nitrogen gas/or evaporator (the rest was kept for further extraction or for identification of other components). The sample was dissolved in 250 µl of 30% methanol and vortexed to suspend the dried debris; centrifuged for 2 minutes or until the solution was clear. Two hundred microliters (200 ul) of supernatant was transferred to a glass vial and evaporated under nitrogen to dryness. The residue was re-dissolved in 50µl N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) derivatizing agent in a 2 ml glass GC vial tube. The vials were tightly capped and heated for 1 hour at 80 - 90 °C on a sand heat/bath. Derivatized samples were cooled to RT and then transferred by a glass syringe into a 200µl glass insert and inserted into the same vial and tightly capped. A sample volume of 1 µl was injected into GC column for analysis.

### 5.3.2 GC-MS analysis

The GC-MS analyses were carried on a Varian CP-3800 GC coupled to a CombiPal autosampler (LEAP Technologies, Carrboro, NC, USA) and a Varian 1200L triple quadrupole MS (Varian, Carey, NC, USA). The GC was fitted with a VF-17ms FS, 30 m X 0.25 µm film thickness Factor Four GC column (Varian). Helium was used as the carrier gas at a constant flow rate of 1 ml/min. The analysis was performed under the following temperature gradient program; 2 min isothermal heating at 70°C, followed by 5°C/min oven temperature ramp to 150°C and held for 5 min; then to 300°C at a rate of 10°C/min and a final 3 min heating at 300°C. The system was then temperature equilibrated for 6 min at 70°C prior to the next injection. Electron ionization (EI)-MS spectra were collected at 70 eV and mass spectra were recorded at 4 scans per second with an m/z 100 – 600 scanning range. The metabolite identification was carried out with the Golm, Germany metabolomics library software and the mass spectra of individual chromatographic peaks were compared to a spectral library (Palisade Corporation, Ithaca, NY, USA). For comparative purposes and quantitative analysis, the peak areas of the compounds were normalized by the sample fresh weight and by dividing the peak area of the sample by the peak area of ribitol (internal standard), resulting in relative response ratios for all compounds.

### 5.3.3 Extraction and LC-MS metabolite profiling

LC-MS analysis was carried out following the method by Tohge and Fernie (2010) where an aliquot of frozen tissues (200 mg) was weighed in a 2 ml Eppendorf tube and extracted by adding 5 µl of extraction buffer (80% methanol solution prepared using ultrahigh-grade methanol and distilled water) per mg of frozen plant material. One metal ball was then added for homogenization and homogenized with the mixer mill for 2 min. The sample was then centrifuged for 10 min at 12,000 r.p.m and the supernatant transferred to a nanosep centrifugal filter 45 µm (Pall, cat. no. 87900A). It was then centrifuged again for 2 min at 4,000 r.p.m. The supernatant was then transferred to a new Eppendorf tube.

### 5.3.4 LC-MS analysis

The LC-MS analyses were carried on a linear ion trap (IT) ESI–MS system Finnigan Ltq (Thermo Finnigan), HPLC Surveyor System (Thermo Finnigan), analytical column Luna C18 (2), 2.0-mm diameter, 150-mm length, 100-Å pore size and spherical particles of 3 µm (Phenomenex, cat no. 00F-4251-B0) and Xcalibur software (Thermo Finnigan). The m/z calibration was performed by infusion injection of MRFA solution using automatic calibration application in Xcalibur. 50 µl of the solution was transferred to a glass vial for HPLC and refrigerated before use. At least five injections each of 5-µl extraction buffer were performed before the first sample analysis. The whole-matrix sheet of detected peaks was made and the detected peaks identified and annotated.

### 5.3.5 Data Analysis

The relative response ratios for all compounds and accessions were used for data analysis. Three independent biological replicates were used per analysis and the results were expressed as mean values ± standard deviation. Analysis of variance (ANOVA) was conducted using sample, treatment and stage of development as factors and Duncan's test ($P \leq 0.05$) was used for mean comparison and separation. All statistical analysis was carried out by GenStat discovery 14$^{th}$ Edition. Principle Component Analysis (PCA) was performed using DARwin version 6 software on the relative response ratios data to visualize general clustering, trends and outliers among the samples on the scores plot. Unsupervised PCA to separated samples into clusters based on treatment and developmental stages was done using MetaboAnalyst software (Xia and Wishart,

2011). Data processing and multivariate analysis was also performed using XCMS online (The Scripps Research Institute, La Jolla, California, USA) software, which is found online at https://xcmsonline.scripps.edu/. This software allows peak alignments, matching and comparison. The GC–MS files were first converted into netCDF files using the File Converter tool. Files were arranged in one folder that was set as the file source. For the LC-MS data, the whole-matrix sheet of detected peaks was made and the detected peaks identified and annotated. Metalign was used to analyze the binary RAW file.

## 5.4 RESULTS

### 5.4.1 Metabolite profile of the African eggplant leaf tissues

#### 5.4.1.1 Detected metabolites

The GC-MS metabolomic approach characterized compositional changes occurring in the African eggplant leaves during development. In total, 29 compounds were successfully detected and identified using the Golm GC-MS metabolomics library software as shown in Table 5.1. In some cases, the metabolites were not identified from the metabolite library. The detected compounds were categorised into three main groups; organic acids (13), sugars (8) and amino acids (7). Phosphoric acid was also identified in the samples. The results for each compound and individual African eggplant accession were presented as relative response ratios (Figure 5.4, 5.5 and 5.6) obtained by dividing the sample peak area by the peak area of the internal standard, ribitol. The different leaf tissue profiles were quite diverse, and it was also evident that, in all tissues, marked changes in metabolites occurred during ripening of the fruit.

Table 5.1: Metabolites identified from the African eggplant leaf extracts by GC-MS

| Organic acids | Sugars | Amino acids | Others |
|---|---|---|---|
| Isocitrate | Sucrose | Serine | Phosphoric acid |
| Citric acid | Glucose | Proline | |
| Maleic acid | Sorbose | Alanine | |
| Quinic acid | Fructose | Glycine | |
| Ribonic acid | Mannose | Isoleucine | |
| Fumaric acid | Trehalose | Aspartic acid | |
| Glyceric acid | D-Xylose | Glutamic acid | |
| Threonic acid | Myo-inositol | | |
| Manoonic acid | | | |
| D-Heptonic acid | | | |
| Butanedioic acid | | | |
| Hexadecanoic acid | | | |
| 2-Butenedioic acid | | | |

#### 5.4.1.2 Cluster analysis of metabolites in African eggplant leaf tissues

The heatmap represents the mean abundance of metabolites in different fruit samples. The differences in metabolite levels in drought stressed crops were correlated with the levels from the controls and presented by heatmap (Figure 5.1). Based on the correlation analysis, groups of metabolites were recognized that showed similar patterns as a function of developmental stage and stress. The compounds were clustered into four groups, represented in marked blocks (Figure 5.1). Block 1 indicates a group of metabolites that showed an increase in levels with stress, (sucrose, fructose, mannose, xylose, trehalose, isoleucine, malic acid, fumaric acid, citric acid, isocitric acid, glutamic acid and proline); block 2 indicates a group of metabolites that showed a decrease in levels during stress, (glycine, alanine, quinic acid, aspartate, maleic acid, glucose and myo-inositol); block 3 indicates metabolites that showed no significant change ($p > 0.05$), (glyceric acid, hexadecanoic acid, 2- butenedioic acid) while block 4 indicates metabolites that were detected at low levels in most accessions (sorbose, ribonic acid and D-heptonic acid). This analysis clearly positioned the different developmental stages and treatments in a chronological order with replicate samples positioning closely together.

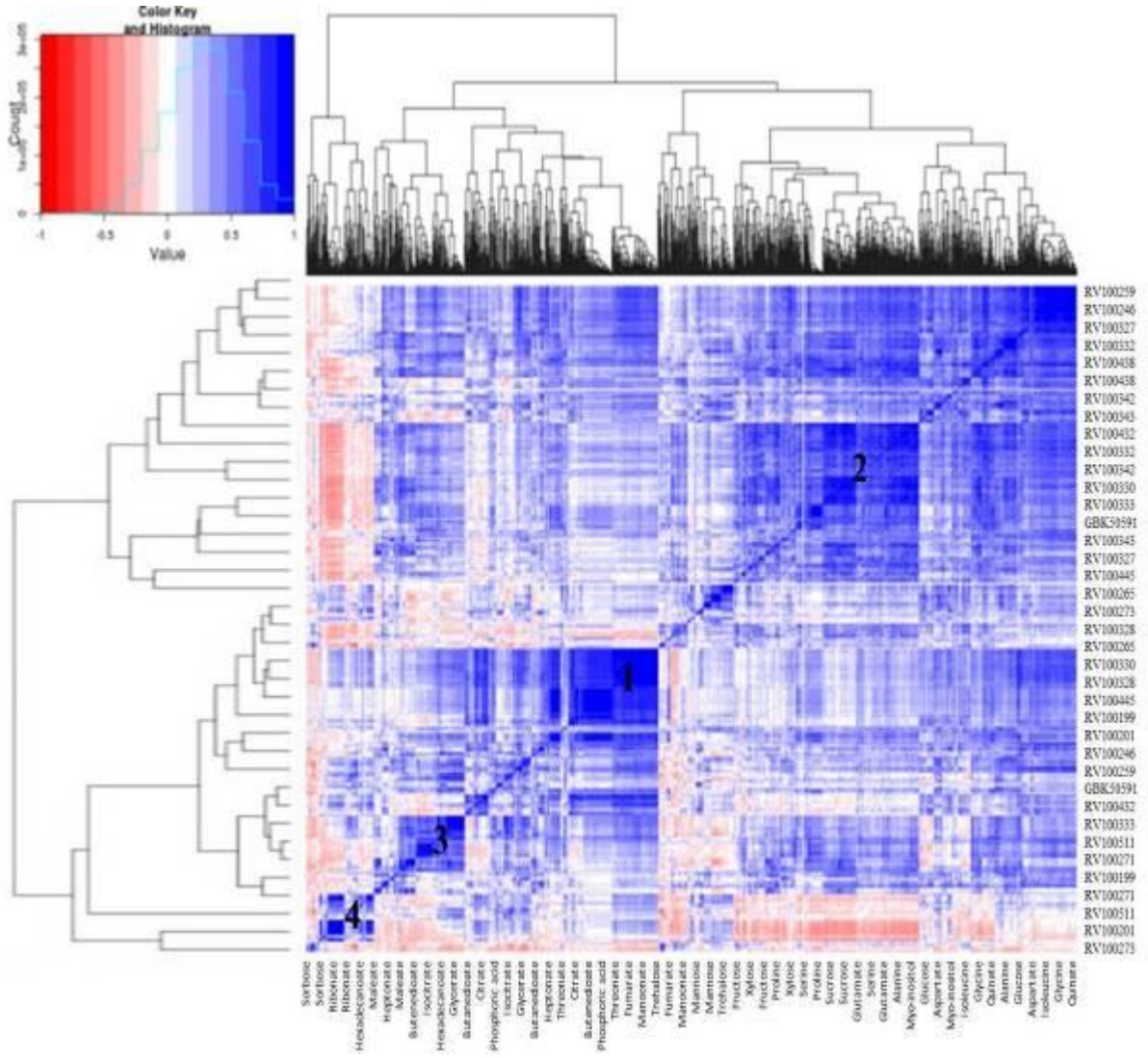



Figure 5.1: Combined cluster analysis of the metabolites of African eggplant leaf samples. Heatmap showing correlation and cluster analysis of metabolite levels in leaves of different stressed and controlled African eggplant accessions at different growth and development stages. Samples were analyzed by GC-MS and the dataset comprised a total of 29 named metabolites. The marked blocks refer to strongly correlated metabolites that show a similar behavior and the colour key indicate the extent of changes in metabolites

Application of PCA to the whole data set using identified metabolites, aimed at finding the directions that best explain the variance in the data set (Figure 5.2). Principal component 1 (PC1) and principal component 2 (PC2) explained 72.4% and 15.5% of total variance (Figure 5.2). According to the PCA, there was an obvious separation between samples within treatments, developmental stages and the accessions and six clusters of samples were observed; drought stressed (0 week, 2 weeks and 4 weeks) and control treatments (0 week, 2 weeks and 4 weeks).

In addition there was discrimination of metabolites between the accessions, drought stressed and control crops as well as at different developmental and growth stages. The response of metabolites to drought stress varied with stage of development and accession (Figure 5.2). There were 12 metabolites and 8 accessions with a significant change ($p < 0.05$) in leaf metabolite content under drought stress. From the PCA, the further a variable is located from the axis origin (loading) the more influential is the variable in discrimination between the different treatments and developmental stages. Stress treatments were characterized by high levels of sucrose, fructose, glucose and trehalose (Figure 5.3), proline, glutamic acid (Figure 5.4) and tricarboxylic acid (TCA) cycle metabolites such as citric acid, isocitric acid, fumaric acid and malic acid (Figure 5.5). This is shown by loadings for variables far from the axis and this indicates metabolites that have a high discriminating power. The data reveals that week 4 of growth (red) displayed the greatest metabolic response to drought stress. According to the PCA plot proline, sucrose, fructose, citric acid and fumaric acid are strongly correlated to the fourth week of drought stress.

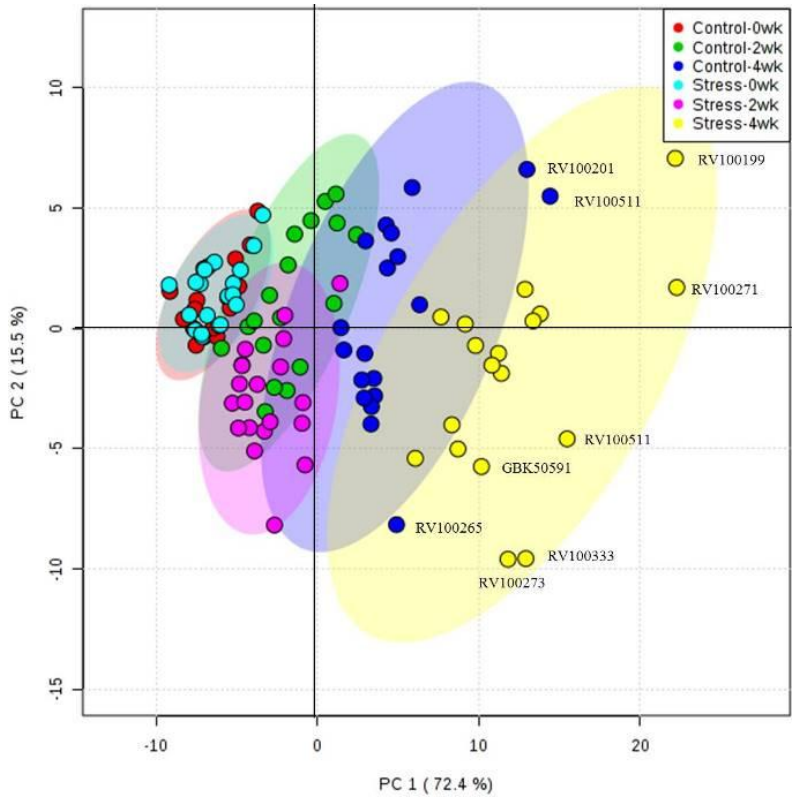



Figure 5.2: Principal component analysis showing cluster of African eggplant accessions at different growth stages. The colors indicate the ripening stages: red (control 0 weeks), green (control 2 weeks), blue (control 4 weeks), light blue (drought stress 0 weeks), pink (drought stress 2 weeks) and yellow (drought stress 4 weeks).

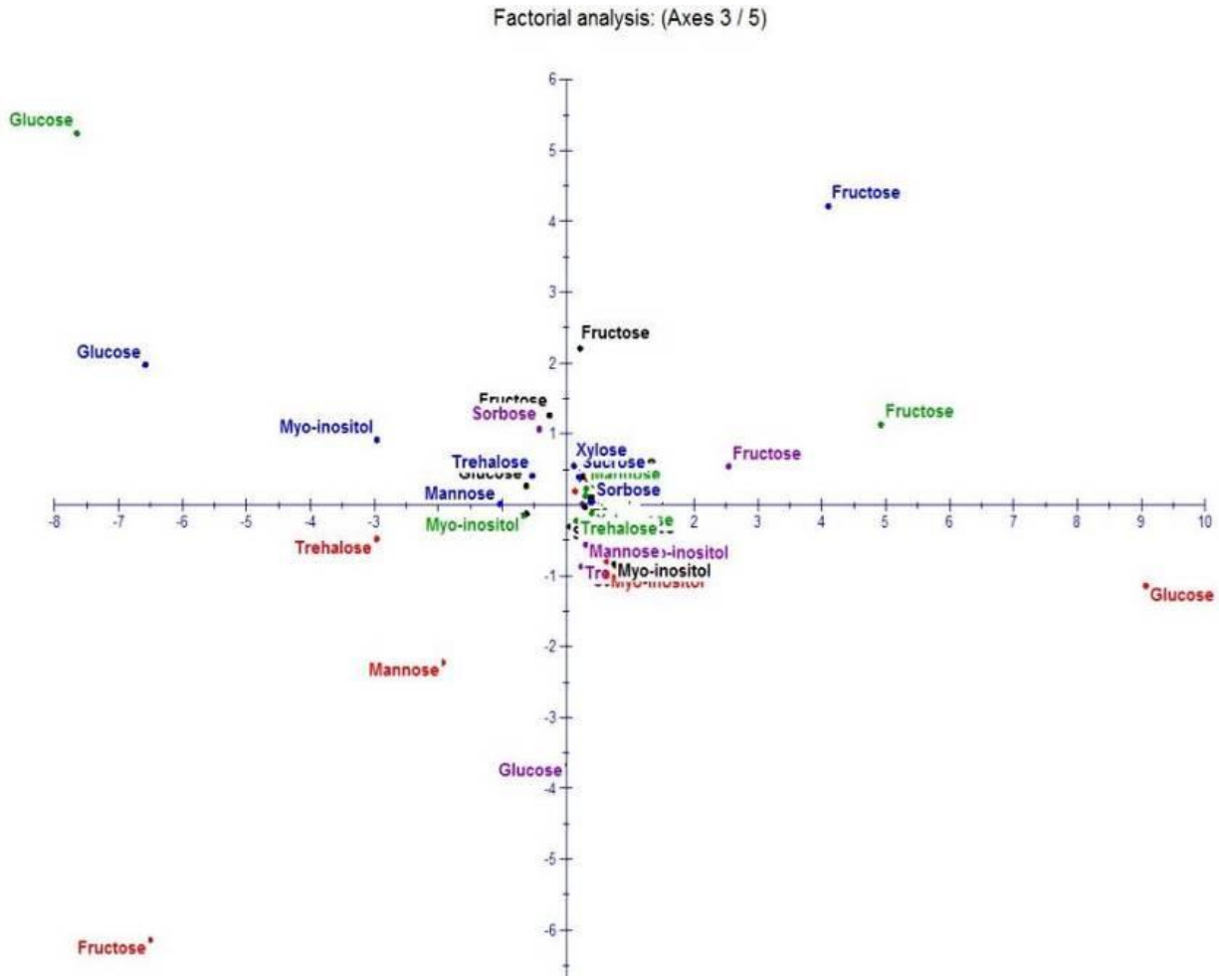



Figure 5.3: Principal component analysis showing distribution of sugars in African eggplant leaves under control and drought stress conditions. Colours represent, Black - 0 weeks stress and control; Purple - 2 weeks stress; Green - 2 weeks control; Red - 4 weeks stress; Blue- 4 weeks control.

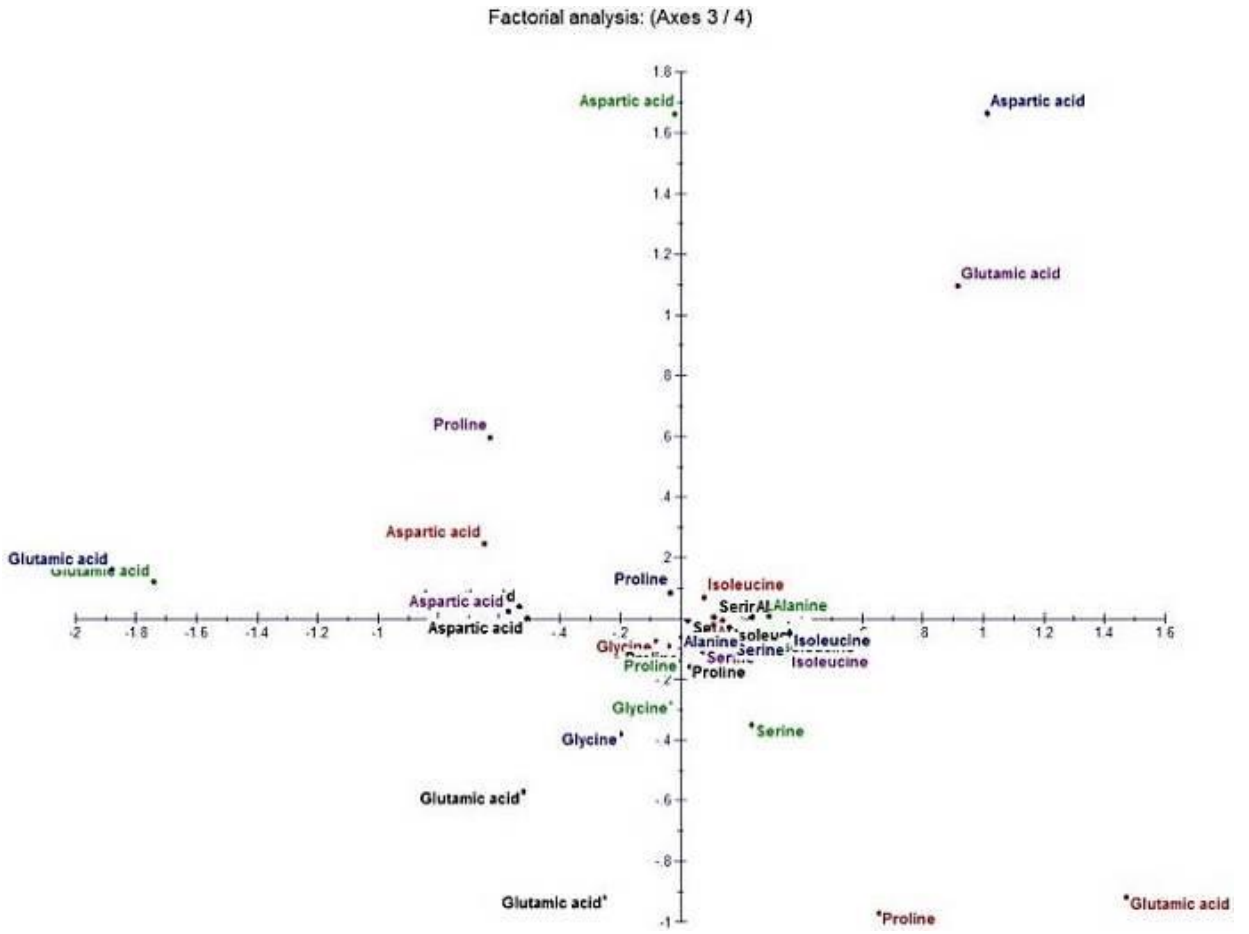



Figure 5.4: Principal component analysis showing distribution of amino acids in African eggplant leaves under control and drought stress conditions. Colours represent, Black - 0 weeks stress and control; Purple - 2 weeks stress; Green - 2 weeks control; Red - 4 weeks stress; Blue- 4 weeks control.

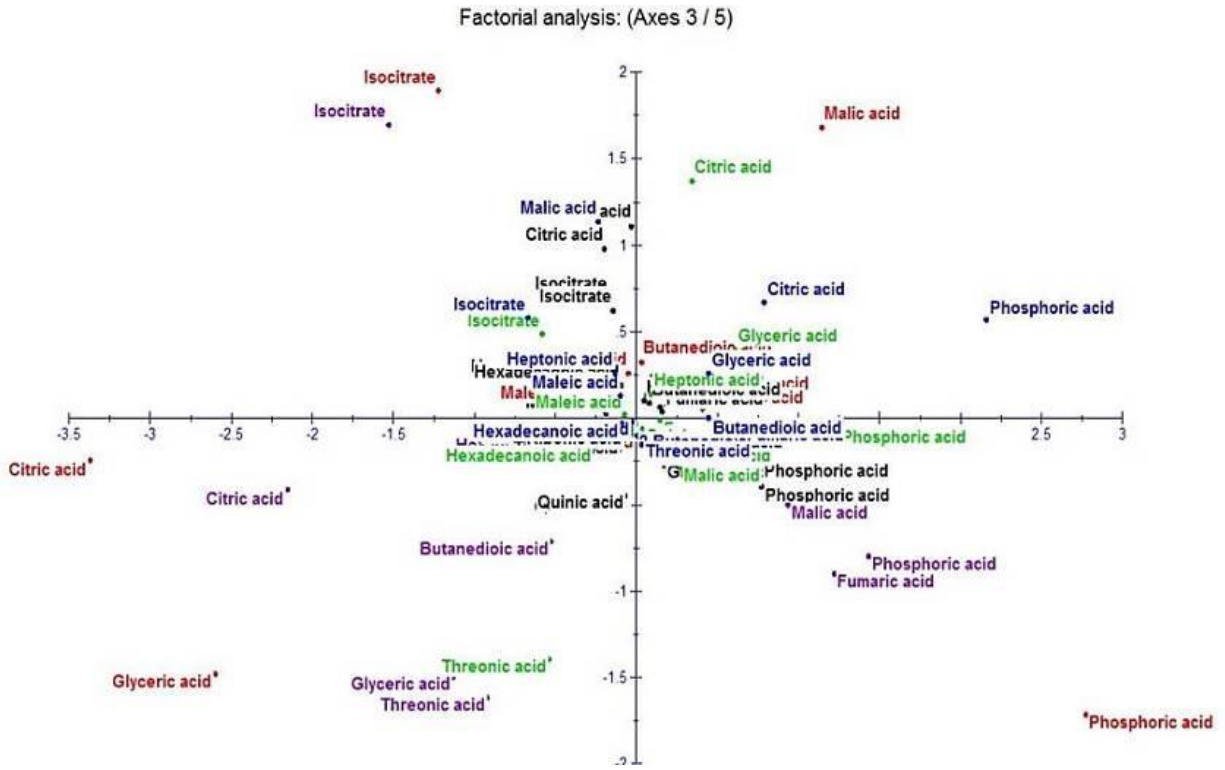



Figure 5.5: Principal component analysis showing distribution of organic acids in African eggplant leaves under control and drought stress conditions. Colours represent, Black - 0 weeks stress and control; Purple - 2 weeks stress; Green - 2 weeks control; Red - 4 weeks stress; Blue- 4 weeks control.

It should be noted that, there were varied contents of the compounds with drought stress and with progressing plant age, with four week old plants after stress (the oldest examined) being distinct. Metabolomics data showed a greater difference between the stage of development and treatment than between distinct accessions, as reflected in the associated PCA (Figure 5.2). On the basis of individual accessions, RV100332, RV100445, RV100259, RV100342, RV100265 and RV100438 were the closest accessions that clustered together and showed the least significant differences in terms of metabolites. These accessions under stress clustered close to the control samples followed by RV100327 RV100511, RV100432, RV100246, RV100328, RV100330 and GBK50591 whilst, RVI00343, RVI00199, RVI00201, RVI00333, RV100271, RVI00273 and RVI00432 were clustered apart from control and were the most distinct. The range of response to stress in terms of wilting and metabolite composition generally varied according to these accessions. This suggested different degrees of tolerance between the accessions with the distinct

accession being more tolerant to drought stress than the other accessions. Drought stress effects were observed for all the accessions through morphological responses and major effects were noted with height and leave sizes of the accessions and this was more characteristic at 4 weeks after stress. Initial morphological characteristics and metabolomic profiling of the accessions uncovered putative metabolite components of the mechanisms responsible for an enhanced tolerance to drought stress.

#### 5.4.1.3 Effect of drought stress on sugar levels in the leaves of African eggplant accessions

ANOVA was used to compare the overall variation in sugar composition associated with drought stress at different stages of growth and development. There was significant difference ($p < 0.05$) between the three growth stages; 0, 2 and 4 weeks, whereby an increase in the levels of sugars was observed during growth (Figure 5.6). The sugar levels of individual accessions were observed to follow the same trend as the averages reported in Figure 5.6. The major sugars observed in the African eggplant species were glucose, fructose, sucrose, trehalose and myo-inositol; whereas mannose, xylose and sorbose were in low concentration. The results of this study revealed varied response of sugars to drought stress between the African eggplant accessions under drought stress and during plant growth and development. For instance, the levels of sucrose, glucose, fructose, xylose, mannose and trehalose with exception of myo-inositol which accumulated. On the other hand, sorbose levels was significantly high ($p < 0.05$) in week 2 as compared to week 4, with the control plants displaying significantly lower ($p < 0.05$) levels within the first two weeks but no detection at week 4.

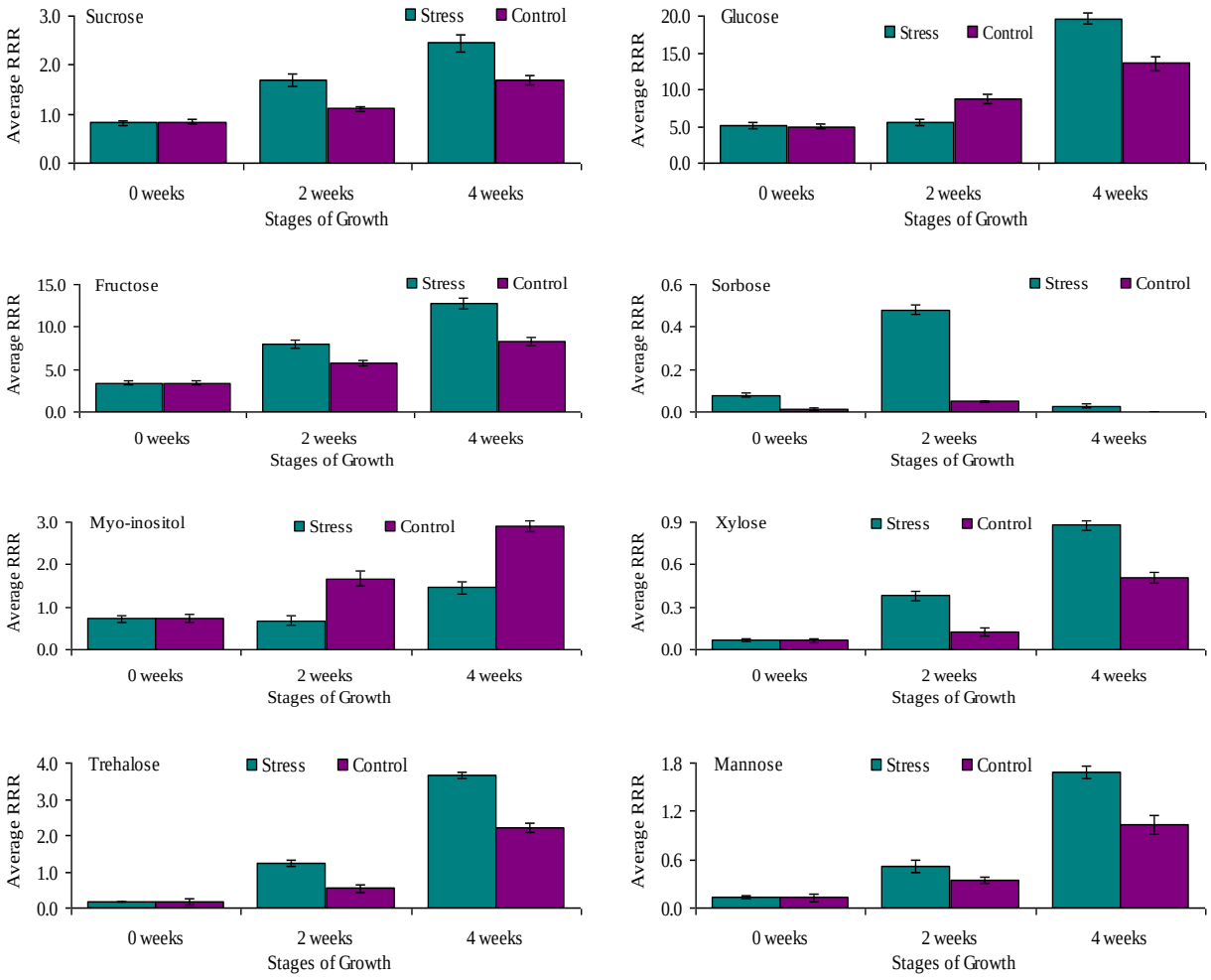



Figure 5.6: Average relative response ratios of sugars in the African eggplants accessions at different growth and development stages during stress. Mean ± SD (n=19)

### 5.4.1.4 Effect of drought stress on Amino acid levels in the leaves of African eggplant accessions

Amino acids are precursors for the synthesis of important proteins and their accumulation in crops defines their nutritional importance. From this study, the amino acid levels of African eggplant is shown in Figure 5.7 and indicates varying levels of amino acids in the stressed and control plants as well as at different growth and developmental stages from 0 – 4 weeks.

The leaves were characterized by relatively high accumulation of amino acids; aspartic acid, glutamic acid and proline (Figure 5.7) in all the African eggplant accessions. However, there was a significant increase ($p < 0.05$) in proline and glutamic acid under drought stress. These

compounds reported relative response ratios ranging from 0.3 – 5. On the other hand, significantly lower levels of glycine, serine, isoleucine and alanine were observed in the leaves of all accessions (Figure 5.5).

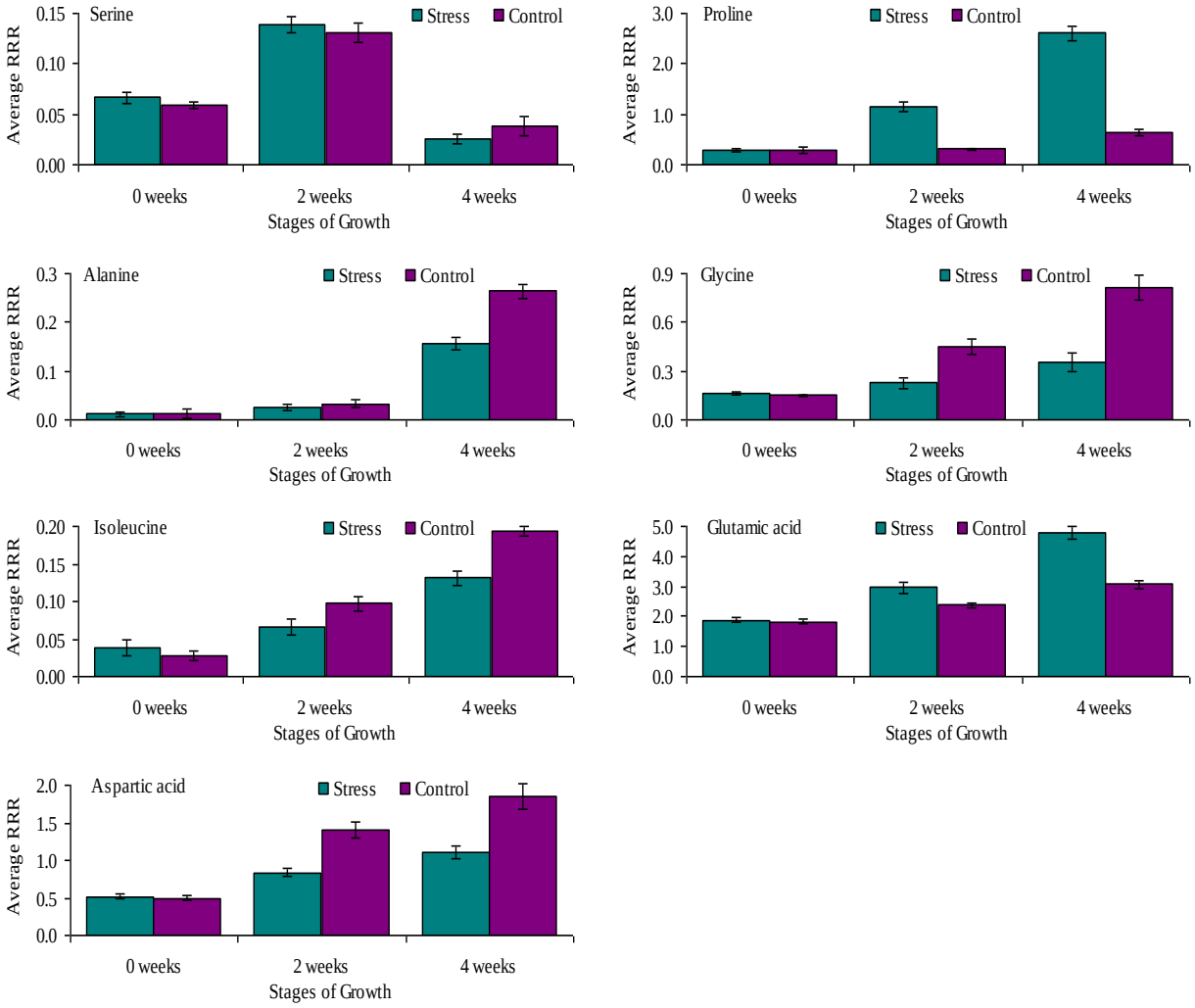



Figure 5.7: Average relative response ratios of amino acids in the African eggplants accessions at different growth and development stages during stress. Mean ± SD (n = 19).

With regard to the free amino acids in the leaves the levels of proline and glutamic acid significantly increased ($p < 0.05$) during drought stress conditions. An increase in proline and glutamic acid in the leaves at week 4 suggests an increased assimilation at the mature stage. On the other hand, serine showed a significant increase ($p < 0.05$) at the second week of growth but decreased at week 4. No significant difference ($p > 0.05$) was observed in serine level between the drought stressed and the control accessions. On the contrary, the contents of isoleucine, alanine, glycine and aspartic acid showed a significant decrease ($p < 0.05$) in the

drought stressed crops in all the accessions. In addition, the levels of the following amino acids were significantly high ($p < 0.05$) in the following accessions as indicated by large relative response rations: proline (RV100273 and RV100432), glycine (RV100201, RV100445 and RV100199), aspartic acid (RV100271, RV100333, RV100265 and RV100342), glutamic acid (RV100343, RV100199, RV100332 and RV100511), alanine (RV100332, RV100265 and RV100445), under drought stress.

#### 5.4.1.5 Effect of drought stress on organic acid levels in African eggplant accessions leaves

GC-MS spectra were generated for organic acids and the results are shown in Figure 5.8. Comparison of the organic acids revealed no differences between the stressed and the control tissues although the quantitative responses of most compounds and accessions varied. As would be expected the major organic acids in leaves of all the African eggplant accessions were citric acid, glyceric acid, malic acid, fumaric acid and isocitric acid as well as an inorganic acid, phosphoric acid. They mainly contributed to the differences in total organic acids. Citric acid, phosphoric acid and glyceric acid were the major acids, followed by malic acid, fumaric acid and isocitric acid and other less abundant acids (Figure 5.8). An important feature of organic acids is that they are the main intermediates of photosynthesis. Most of these products are also important as they serve as precursors for the biosynthesis of amino acids as shown in the pathway (Figure 5.9). These TCA cycle metabolites together with phosphoric acid were significantly increased ($p < 0.05$) in the stressed crops as compared to the controls, indicating that energy production in TCA cycle was enhanced under drought stress. Meanwhile, maleic acid content decreased in the leaves of drought stressed plants as compared to control plants in all the accessions (Figure 5.8). In addition, the stress response of the young leaves and mature leaves were distinct with week 4 samples reporting significantly higher ($p < 0.05$) levels of most of the metabolites. Considerably lower levels were observed in butenedioic acid, maleic acid and hexadecanoic acid. In contrast, no significant differences ($p > 0.05$) were observed in the levels of hexadecanoic acid, butanedioic acid, quinic acid and glyceric acid in the leaves of control and stressed accessions. Ribonic acid reported significantly lower ($p < 0.05$) levels in all the accessions with relative response ratios ranging between 0.0016-0.028 as compared to other organic acids. The level was significantly lower ($p < 0.05$) or not detected in the 2 weeks stage of most accessions.

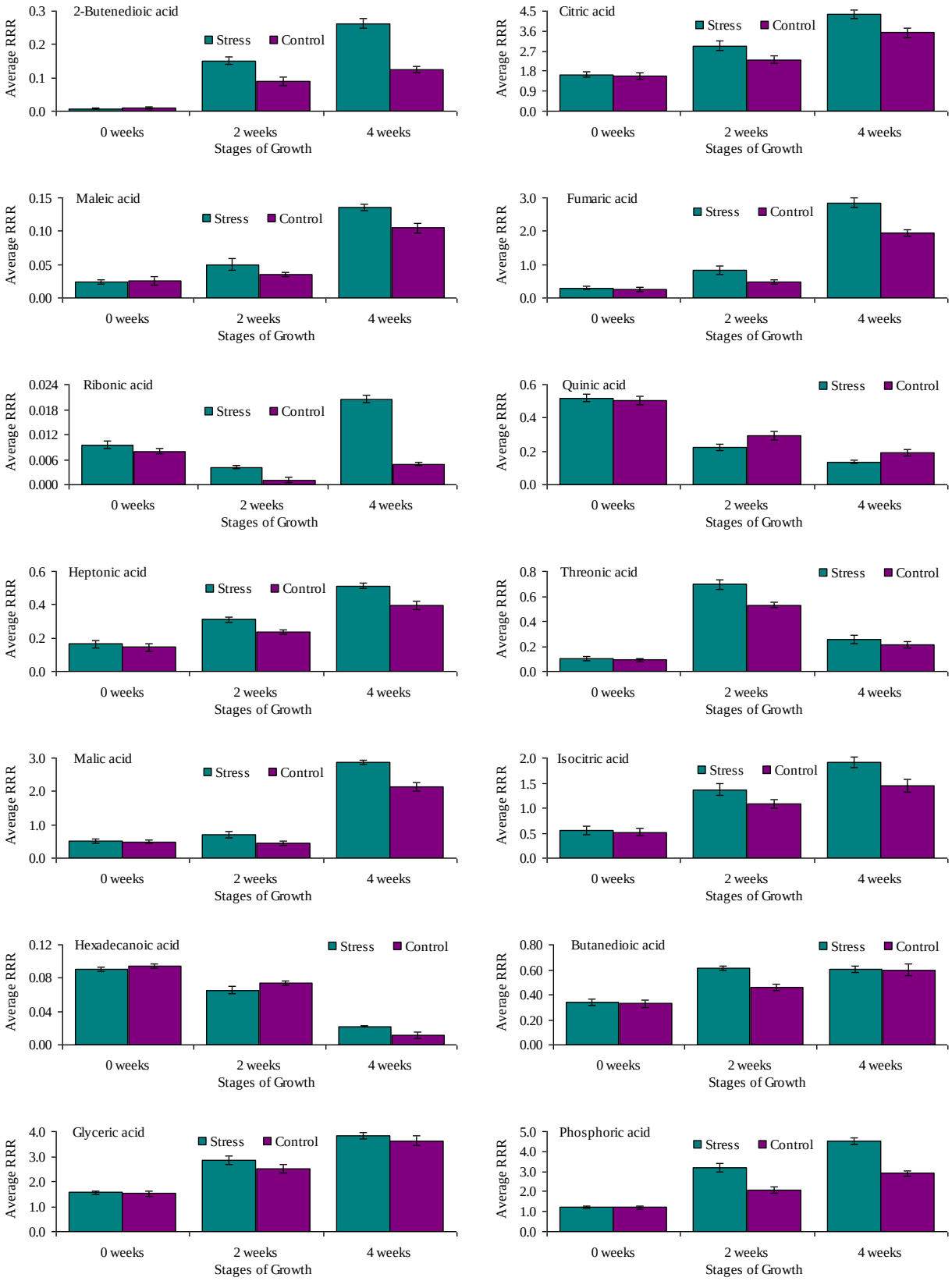



Figure 5.8: Average relative response ratios of organic acids in the African eggplants accessions at different growth and development stages during stress. Mean ± SD (n = 19).

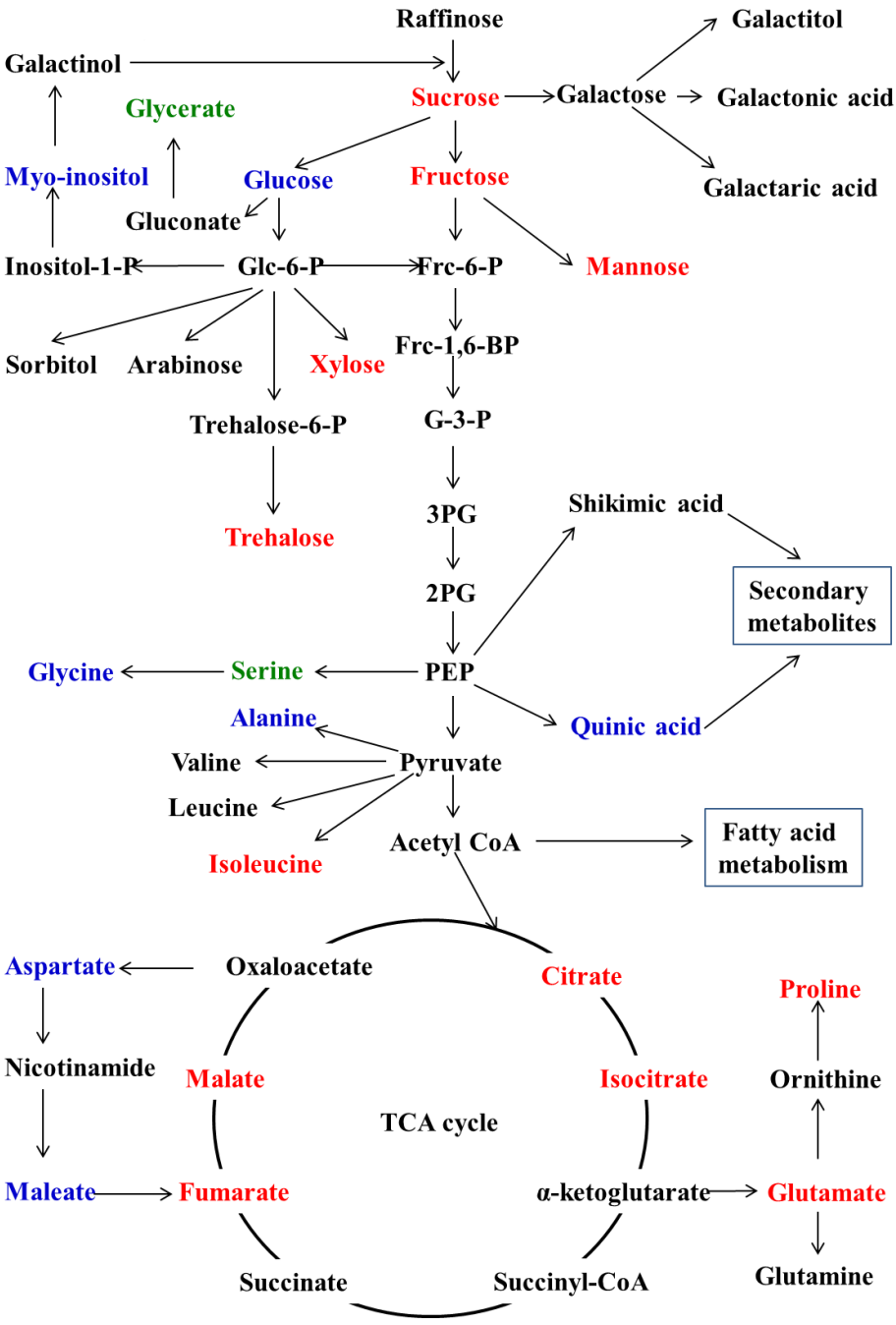



Figure 5.9: Metabolic pathway indicating the connections of carbohydrate and protein metabolism in plants. The metabolites associated with the metabolism of various identified sugars, amino acids and organic acids in the African eggplant accessions during stress and at different growth and development stages. Metabolites written in (black) were not detected, (green) were detected but were not significantly affected by drought stress, (red) indicate metabolites with greater relative response ratios (relative abundance) in drought conditions, (blue) indicate metabolites with lower relative abundance in the stress.

### 5.4.2 Metabolite profile of the African eggplant fruit tissues

GC-MS-based metabolite profiling was performed in order to gain insight into the metabolic changes that occur in African eggplant fruits under stress. To explore metabolic changes caused by drought stress, the fruits were compared at different ripening stages under control and stress conditions. The data was normalized before analysis (Figure 5.10) to allow general-purpose adjustment for differences among samples.

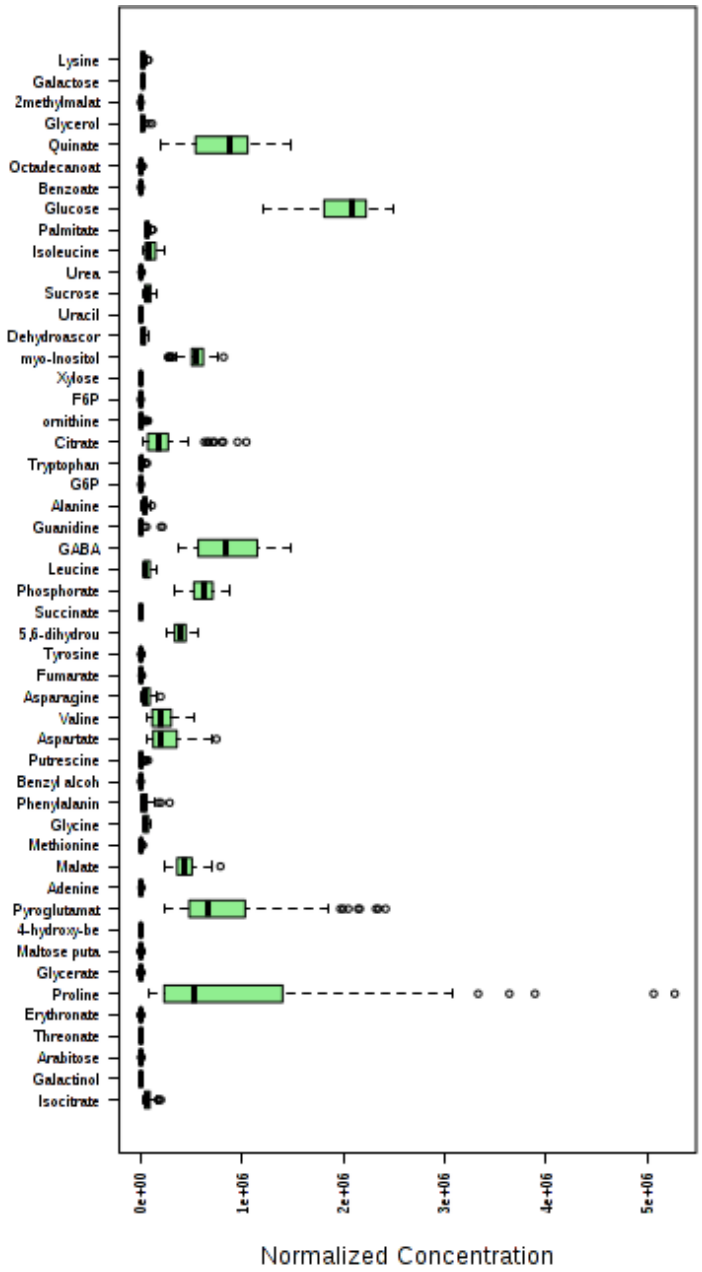



Figure 5.10: Box plots and kernel density plots after data normalization. The boxplots show at most 50 features due to space limit and the density plots are based on all samples. Selected methods are row-wise normalization and data scaling.

#### 5.4.2.1 Detected metabolites in African eggplant fruits

Table 5.2 provides an overview of the number of metabolites detected from all the accessions in the different ripening stages. A total of 68 metabolites were detected in the fruits and distributed into major compound classes comprising of amino acids and their derivatives (23), organic acids (22), sugars and sugar alcohols (16) and nitrogen (N-) compounds (7) (Table 5.2). Unlike the leaf tissues, the fruit tissues provided comprehensive metabolite patterns. Metabolites in the amino acid and organic acid groups comprised the majority of the metabolites detected in the fruits at the three different stages of ripening. In addition, sugars and sugar alcohols were identified in the fruits together with nitrogen containing compounds. The dominance of these metabolites in the tissues signifies their important role in plant metabolism. To elucidate the significance in metabolite levels among accession, relative quantification was performed by using the relative intensities of the peak areas. The number and abundance of metabolites in the control and stressed accessions varied significantly ($p < 0.05$) between the different ripening stages and accessions. However, metabolites were highly enriched in the mature red fruits followed by breaker and the mature green. Of the 68 metabolites proline, glutamate, $\gamma$-amino butyric acid (GABA), 3-chlorogenic acid, glucose, sucrose, myo-inositol, citrate, quinic acid and ornithine showed a significant increase ($p < 0.05$) on the drought stressed fruits (Table 5.2).

Table 5.2: Compilation of the metabolites identified by GC-MS in the African eggplant fruits

| **Sugars and sugar alcohols** | **Amino acids** | **Organic acids** |
|---|---|---|
| Fructose-6-phosphate | Alanine | Dehydroascorbate dimer |
| Glycerol-3-phosphate | Arginine | 4-hydroxy-benzoic acid |
| Glucose-6-phosphate | Aspartate | 3,4-dihydroxybenzoate |
| Raffinose | Glutamate | 3-chlorogenic acid |
| Arabitose | Glutamine | 2-methylmalate |
| Galactose | Asparagine | Octadecanoate |
| Fructose | Methionine | 2-oxyglutarate |
| Glucose | Phenylalanine | Erythronate |
| Maltose | Tryptophan | Nicotinate |
| Sucrose | Isoleucine | Threonate |
| Xylose | Threonine | Glycolate |
| Glycerol | Histidine | Succinate |
| Erythritol | Tyrosine | Palmitate |
| Galactinol | Leucine | Benzoate |
| Myo-inositol | Glycine | Glycerate |
| Benzyl alcohol | Proline | Isocitrate |
| | Lysine | Fumarate |
| **N-compounds** | Valine | Pyruvate |
| 5,6-dihydrouracil | Serine | Malate |
| Ethanolamine | GABA | Quinate |
| Putrescine | Ornithine | Citrate |
| Guanidine | Pyroglutamate | Phosphorate |
| Adenine | 4-hydroxyproline | |
| Uracil | | |
| Urea | | |

### 5.4.2.2 Correlation of the GC-MS metabolites in African eggplant fruits

Correlation analyses between all metabolite pairs were done using Pearson's correlation (Pearson's $r$) and the results presented in Figure 5.11. The different accessions showed similar metabolite responses when subjected to drought stress conditions (Figure 5.11) although some of the metabolites showed no correlation.

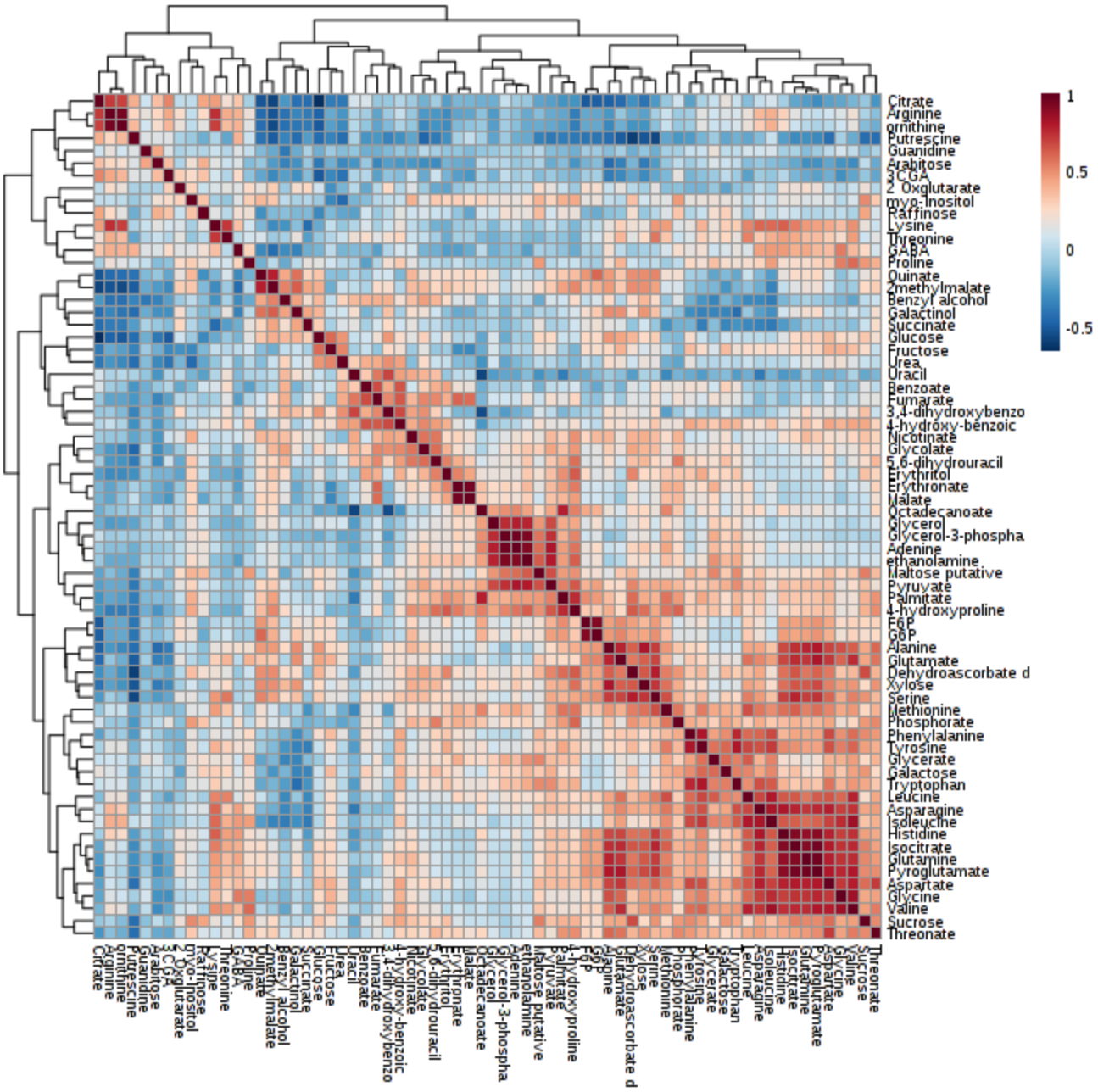



Figure 5.11: Pearson product-moment correlation analysis of African eggplant fruits metabolites measured in ripening stages at different treatments. Red color indicates positive correlation and blue indicates negative correlation.

Besides the clear correlation of the metabolites, the general metabolome profile of the African eggplant fruit in the course of the progressive ripening was also seen. Heatmap (Figure 5.12, Figure 5.13 and Figure 5.14) showed significant accumulation of metabolites in the mature red and breakers stage of stressed fruits. On the other hand, the mature red fruits of the control fruits

had similar trend but not significantly high ($p < 0.05$). It is evident that fruit metabolites accumulated slowly during the early stage of ripening and dramatically increased during the last stage when the crops were experiencing drought stress.

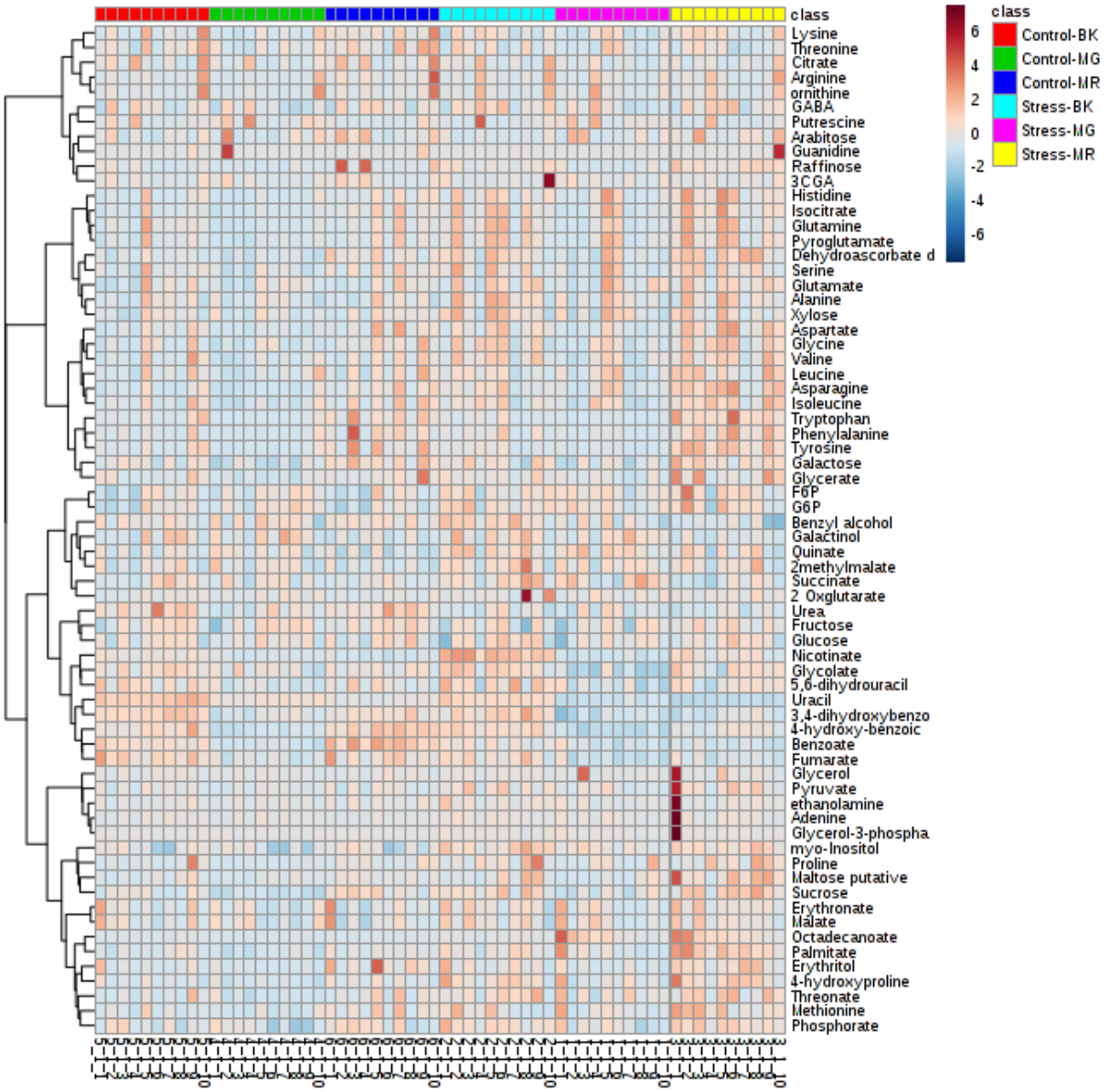



Figure 5.12: Heatmap representing the distribution of metabolites in individual African eggplant fruits at different ripening stages under different conditions. The colors indicate the ripening stages: green (control mature green), red (control breaker), blue (control mature red), pink (drought stress mature green), light blue (drought stress breaker) and yellow (drought stress mature red). The colour key indicates the extent of changes in metabolites; red color indicates high accumulation and blue indicates low accumulation; (Appendix 2. for accession abbreviations)

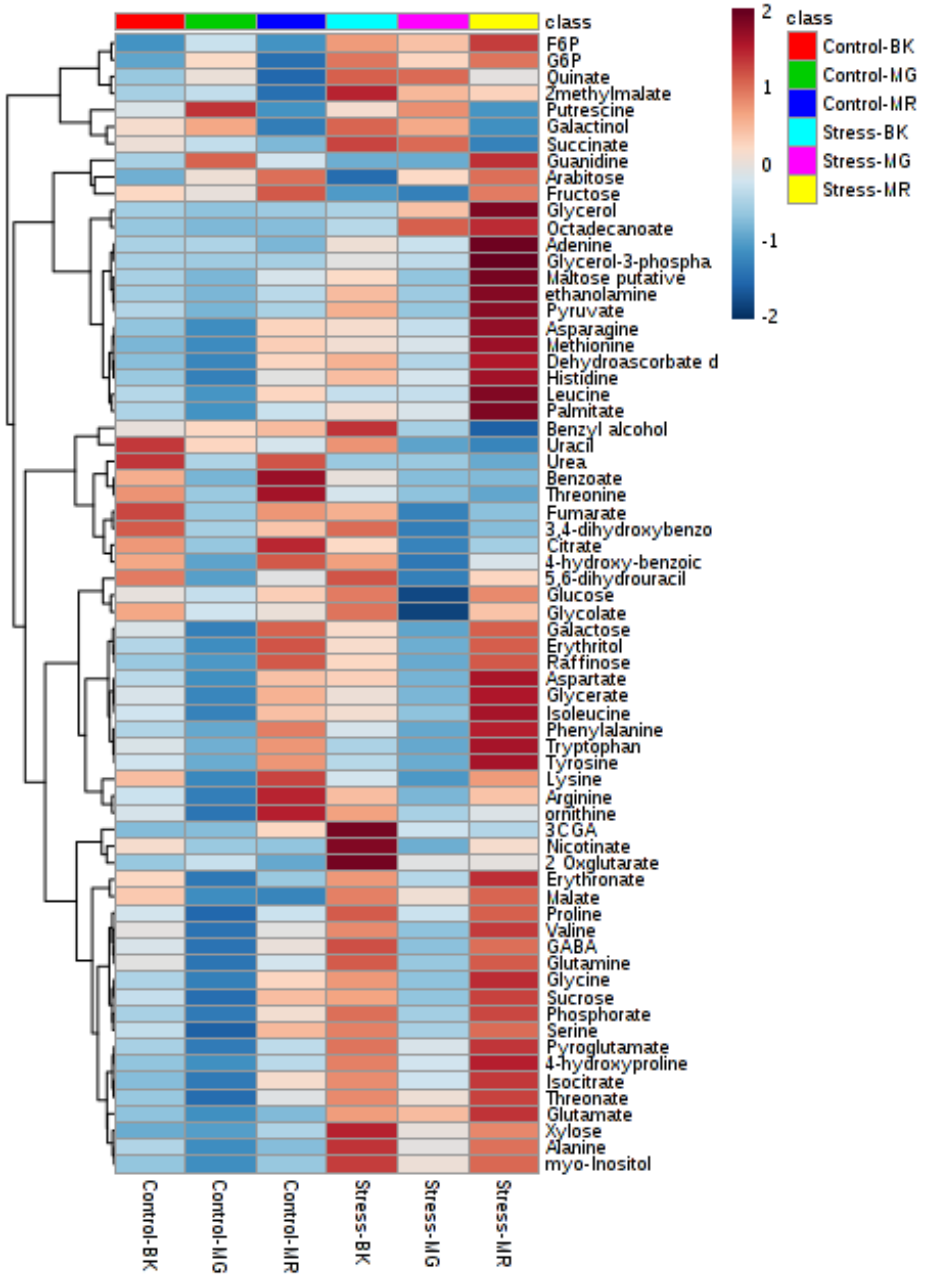



Figure 5.13: Heatmap representing the distribution of metabolites at different ripening stages of African eggplant fruits. The colors indicate the ripening stages: green (control mature green), red (control breaker), blue (control mature red), pink (drought stress mature green), light blue (drought stress breaker) and yellow (drought stress mature red). The colour key indicates the extent of changes in metabolites; red color indicates high accumulation and blue indicates low accumulation; (Appendix 2. for accession abbreviations)

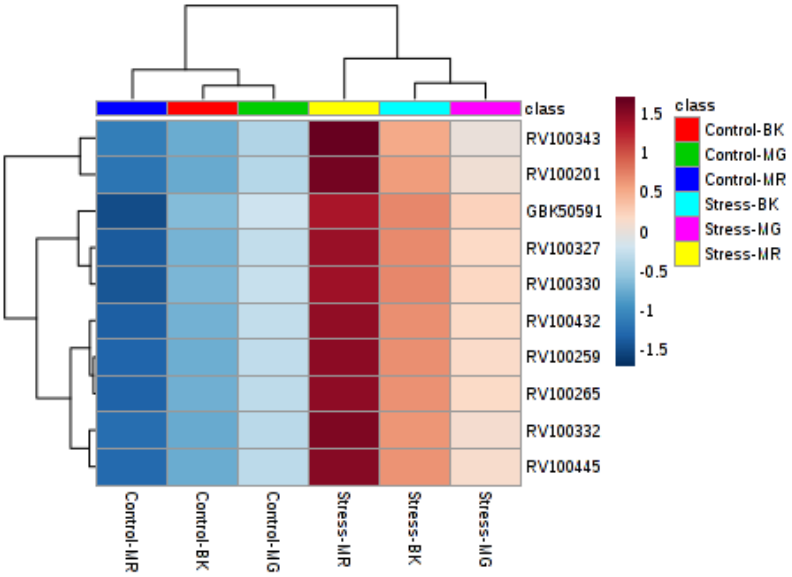



Figure 5.14: Heatmap representing the distribution of metabolites between the different African eggplant fruits and ripening stages. The colors indicate the ripening stages: green (control mature green), red (control breaker), blue (control mature red), pink (drought stress mature green), light blue (drought stress breaker) and yellow (drought stress mature red). The colour key indicates the extent of changes in metabolites; red color indicates high accumulation and blue indicates low accumulation; (Appendix 2. for accession abbreviations)

### 5.4.2.3 Hierarchical cluster analysis

Hierarchical cluster analyses of fruit tissues showed that the mature red of the control and the drought stressed fruits were distinctly separated from the rest. This variation is further explained by the PCA score plot (Figure 5.15) derived from GC-MS data. It is interesting to point out that PCA score and loading plots showed a clear distinction and groupings in metabolites between the ripening stages. In all the accession, the mature ripe fruits showed significantly high ($p < 0.05$) contents of sugars and amino acids. In addition, a clear distinction was observed between the control and drought stressed conditions. According to the PCA results (Figure 5.15), drought stress contributed to a difference in metabolites between the different accessions and distinct responses were observed in RV100265, RV100432, RV100327 and RV100201 accessions under drought stress. Furthermore, cluster analysis clearly separated this group from the other groups based on the accumulation during stress. Variation in the dataset in the different ripening stages can be explained by principle component 1 (PC1) which accounts for 21.2 %, principal component 2 (PC2) with 8.4 % and principal component 3 (PC3) with 7.4 % to the separation (Figure 5.16). The major loadings for metabolites were sucrose, fructose, glucose,

pyroglutamate, 3 chlorogenic acid (3CGA), guanidine, citrate and phenylalanine (Figure 5.17). Since clear discrimination of these metabolites was observed in drought stressed, it indicates that drought stress treatments contributed significantly ($p < 0.05$) to the fruit metabolite changes.

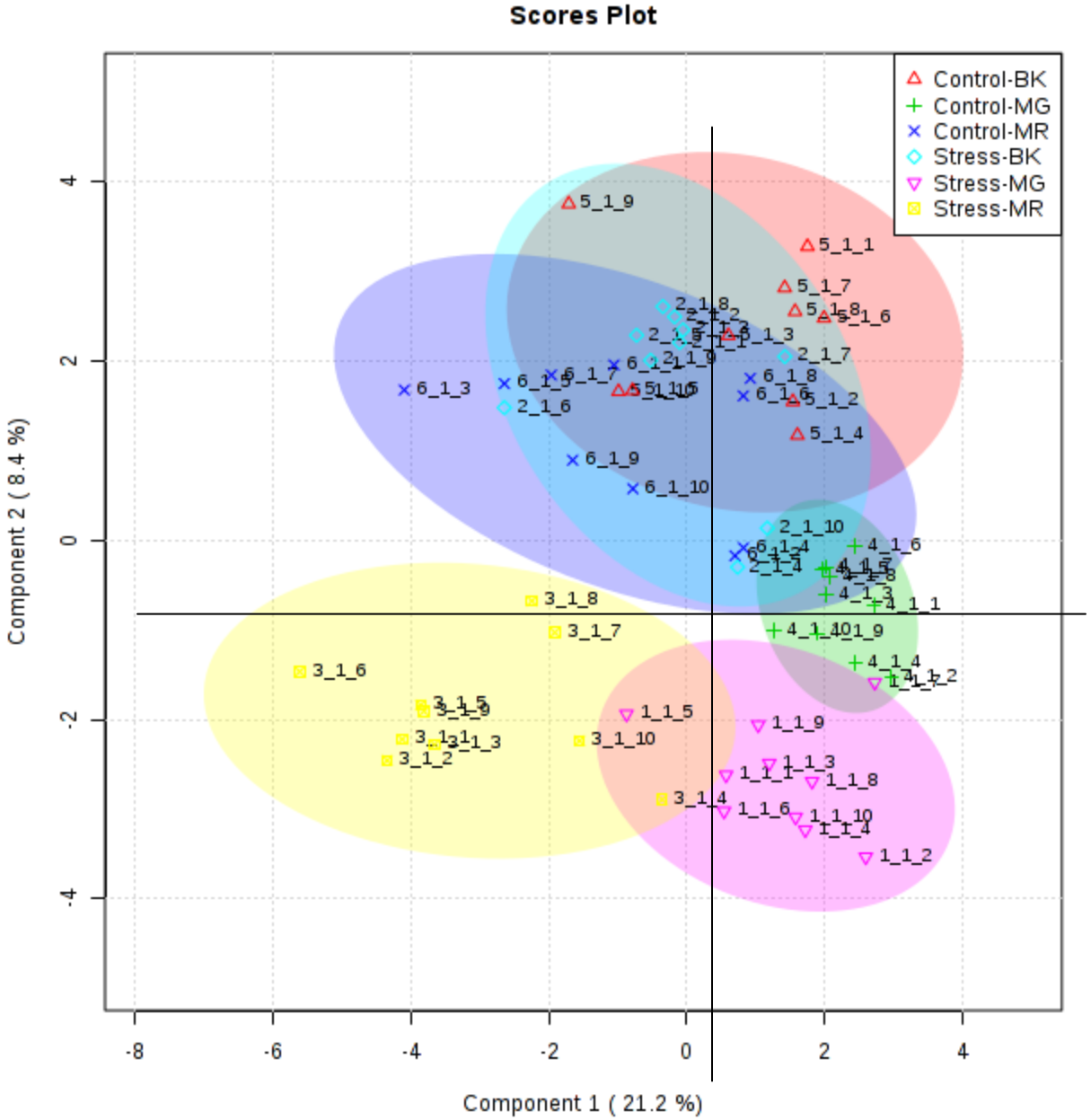



Figure 5.15: Principal component analysis scores scatter plot showing PC1 and PC2 of African eggplant fruits during different stages of ripening based on metabolite profiles obtained from GC-MS (Appendix 2. for accession abbreviations)

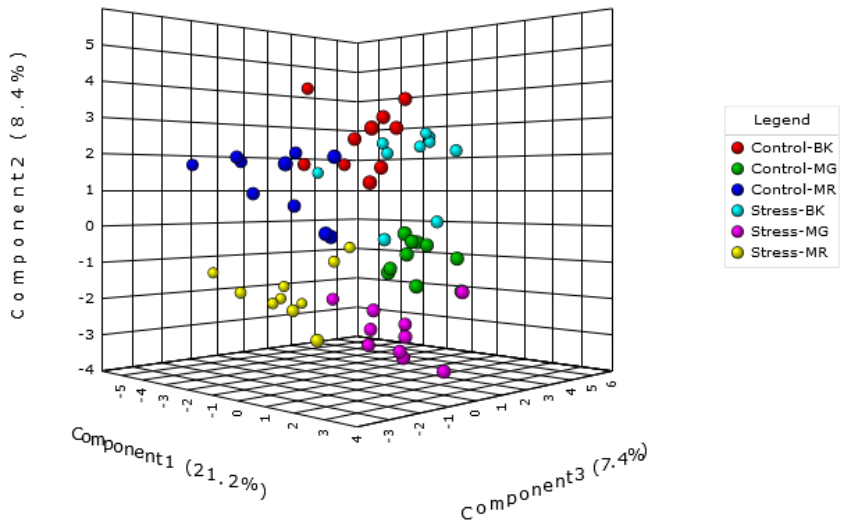



Figure 5.16: Three dimensional principal component analysis scores plot showing cluster of PC1, PC2 and PC3 of control and drought stressed African eggplant fruits during different stages of ripening (Appendix 2. for accession abbreviations)

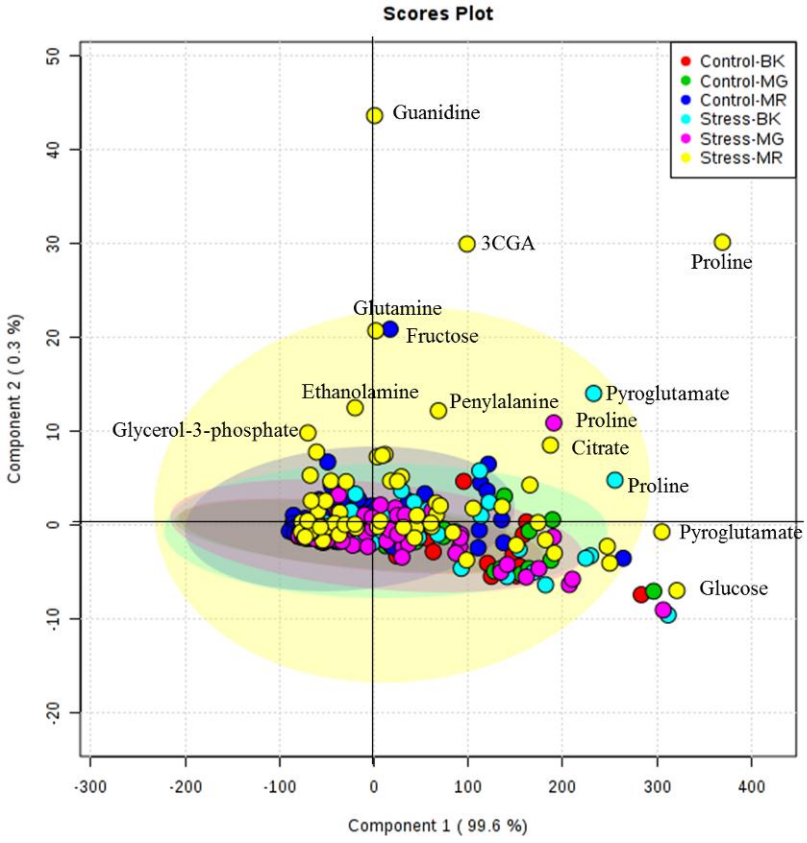



Figure 5.17: Principal component analysis scores scatter plot of metabolite profiles obtained from GC-MS in control and drought stressed African eggplant fruits during different stages of ripening. The colors indicate the ripening stages: green (control mature green), red (control breaker), blue (control mature red), pink (drought stress mature green), light blue (drought stress breaker) and yellow (drought stress mature red). (Appendix 2. for accession abbreviations)

#### 5.4.2.4 Effect of drought stress on metabolite profile of African eggplant fruits

This study found out that various metabolites accumulated significantly (< 0.05) and an important variation in response between the different accessions and the different treatments was observed. The control and drought stress conditions induced clear differences in the metabolite profiles. Due to the large number of metabolites identified, the average of the peak abundance for the different ripening stages was carried out. Table 5.3 shows the average relative content of the selected metabolites calculated based on peak area. In most of the accessions, almost all the metabolites showed progressive accumulation towards the last stage of ripening except for sixteen metabolites that showed an accumulation in breaker stage and then declined at mature red stage. These 16 metabolites were composed of amino acids (alanine, proline and glutamine), sugar alcohols (benzoyl alcohol and myo-inositol), organic acids (fumarate, malate, citrate, glycolate, oxoglutarate, nicotinale, 2 methyl malate, 4 hydroxybenzoate, 3, 4 dihydroxybenzoate) and N-containing compounds (uracil and 5, 6 dihydoxyuracil) (Table 5.3). On the other hand, under control conditions, less metabolites were accumulated. By contrast, under drought stress, levels of majority of the organic acids were present at higher levels in the fruits, including 3CGA, quinate, citrate, malate, isocitrate and GABA. A number of amino acids except ornithine and serine significantly increased ($p < 0.05$) in drought stressed. Majority of the amino acids including asparagine, aspartate and glutamate showed an increase in the contents with ripening stages. Interestingly, some amino acids and derivatives such as alanine, proline, arginine and glutamine and amino acid derivatives pyroglutamate and GABA reported significantly high ($p < 0.05$) levels in the breaker stages. However, for these amino acids, no significant difference ($p > 0.05$) was observed between the contents of the breaker and the mature red stages. However, isoleucine and leucine did not differ significantly between drought stressed and the control accessions. Similarly, sugars particularly glucose, fructose, sucrose and other metabolites; phosphorate, 5,6-dihydrouracil, myo-inositol was also highly abundant in drought stressed fruits. These metabolites have been well documented as the key osmoprotectants in plants during drought stress. Their biosynthesis during drought stress has been reported in fruits and leaves as reported in tomato. It is evident that proline and glutamate levels are sensitive to drought stress and their accumulation is a significant response of plant under drought stress. Furthermore, it was noted that the level of soluble sugars was very specific and dependent not only upon accession but also upon ripening stage. Interestingly, significant higher ($p < 0.05$) levels of

citrate, malate, fumarate, α-ketoglutarate as well as glucose-6-phosphate and fructose-6-phosphate, the immediate precursor of TCA cycle were observed for the fruits of drought-stressed accessions compared to control accessions, thus indicating that glycolysis was enhanced during stressed.

Table 5.3: Average relative responses of the African eggplant fruit metabolites under control and drought stress conditions

| Metabolites | Stress | | | Control | | |
|---|---|---|---|---|---|---|
| | Mature green | Breaker | Mature red | Mature green | Breaker | Mature red |
| Alanine | 4.396E+04 | 5.939E+04 | 5.516E+04 | 3.168E+04 | 3.949E+04 | 3.635E+04 |
| Arginine | 5.682E+02 | 8.534E+02 | 8.419E+02 | 4.542E+02 | 6.881E+02 | 1.082E+03 |
| Asparagine | 5.223E+04 | 6.675E+04 | 1.178E+05 | 2.450E+04 | 4.099E+04 | 7.074E+04 |
| Aspartate* | 1.662E+05 | 2.881E+05 | **4.293E+05** | 1.313E+05 | 2.128E+05 | 3.010E+05 |
| GABA* | 7.850E+05 | **9.950E+05** | **9.721E+05** | 7.060E+05 | 8.454E+05 | 8.643E+05 |
| Glutamate* | 8.562E+05 | **1.216E+06** | **1.338E+06** | 4.807E+05 | 7.422E+05 | 7.948E+05 |
| Glutamine | 9.349E+03 | 1.967E+04 | 1.62E+04 | 4.813E+03 | 1.272E+04 | 1.185E+04 |
| Isoleucine | 7.703E+04 | 1.020E+05 | 1.487E+05 | 5.801E+04 | 9.086E+04 | 1.120E+05 |
| Leucine | 5.848E+04 | 5.842E+04 | 9.214E+04 | 4.631E+04 | 5.685E+04 | 6.717E+04 |
| Lysine | 1.745E+04 | 2.223E+04 | 2.740E+04 | 1.653E+04 | 2.593E+04 | 3.052E+04 |
| Histidine | 2.216E+04 | 3.009E+04 | 4.470E+04 | 8.333E+03 | 1.690E+04 | 2.360E+04 |
| ornithine | 1.124E+04 | 1.379E+04 | 1.208E+04 | 9.347E+03 | 1.197E+04 | 1.553E+04 |
| Proline* | 9.174E+05 | **1.840E+06** | **1.626E+06** | 2.636E+05 | 9.845E+05 | 9.193E+05 |
| Phenylalanine | 1.827E+04 | 4.319E+04 | 9.935E+04 | 1.819E+04 | 3.398E+04 | 7.917E+04 |
| Pyroglutamate* | 1.485E+05 | 1.547E+05 | 1.732E+05 | 1.046E+05 | 1.171E+05 | 1.150E+05 |
| Serine | 6.676E+04 | 8.763E+04 | 8.932E+04 | 5.068E+04 | 6.939E+04 | 8.162E+04 |
| 4-hydroxyproline | 2.757E+04 | 4.033E+04 | 4.733E+04 | 1.702E+04 | 2.244E+04 | 2.575E+04 |
| Methionine | 6.249E+03 | 6.884E+03 | 1.083E+04 | 3.691E+03 | 4.654E+03 | 7.441E+03 |
| Valine* | 1.787E+05 | **2.618E+05** | **2.889E+05** | 1.378E+05 | 2.139E+05 | 2.131E+05 |
| Tryptophan | 2.107E+03 | 6.504E+03 | 2.743E+04 | 2.651E+03 | 9.850E+03 | 1.884E+04 |
| Tyrosine | 8.485E+02 | 1.700E+03 | 5.363E+03 | 8.376E+02 | 2.031E+03 | 3.806E+03 |
| Threonine | 8.484E+03 | 9.257E+03 | 8.118E+03 | 8.604E+03 | 1.068E+04 | 1.197E+04 |
| Glycine | 4.166E+04 | 6.005E+04 | 6.883E+04 | 3.385E+04 | 4.454E+04 | 5.376E+04 |
| Fructose-6-phosphate | 8.328E+02 | 8.606E+02 | 9.177E+02 | 7.658E+02 | 6.883E+02 | 6.870E+02 |
| Glycerol-3-phosphate | 2.692E+02 | 3.650E+02 | 1.055E+03 | 1.958E+02 | 2.159E+02 | 2.094E+02 |
| Glucose-6-phosphate | 2.837E+03 | 3.147E+03 | 3.153E+03 | 2.811E+03 | 2.297E+03 | 2.070E+03 |
| Glucose** | 1.918E+06 | **2.059E+06** | **2.055E+06** | 1.995E+06 | 1.211E+06 | 1.208E+06 |
| Arabitose | 3.223E+03 | 2.371E+03 | 3.616E+03 | 3.144E+03 | 2.685E+03 | 3.612E+03 |
| Galactose | 1.566E+04 | 2.373E+04 | 3.039E+04 | 1.320E+04 | 2.151E+04 | 3.026E+04 |
| Fructose* | 1.530E+05 | 1.570E+05 | **1.909E+05** | 1.075E+05 | 1.179E+05 | 1.194E+05 |
| Maltose putative | 9.975E+02 | 1.648E+03 | 2.901E+03 | 9.052E+02 | 1.111E+03 | 1.371E+03 |

| Metabolites | Stress | | | Control | | |
|---|---|---|---|---|---|---|
| | **Mature green** | **Breaker** | **Mature red** | **Mature green** | **Breaker** | **Mature red** |
| Xylose | 6.773E+02 | 8.388E+02 | 7.679E+02 | 5.664E+02 | 5.787E+02 | 6.248E+02 |
| Sucrose | 5.557E+04 | 9.155E+04 | 1.086E+05 | 3.426E+04 | 6.552E+04 | 8.619E+04 |
| Raffinose | 2.010E+02 | 4.786E+02 | 7.031E+02 | 1.638E+02 | 2.743E+02 | 7.024E+02 |
| Myo-Inositol* | 5.495E+05 | **6.508E+05** | **6.093E+05** | 4.535E+05 | 4.938E+05 | 4.690E+05 |
| Erythritol | 5.173E+02 | 8.961E+02 | 1.248E+03 | 4.065E+02 | 6.776E+02 | 1.274E+03 |
| Benzyl alcohol | 2.561E+03 | 2.804E+03 | 2.426E+03 | 2.659E+03 | 2.629E+03 | 2.068E+03 |
| Glycerol | 1.877E+04 | 1.413E+04 | 2.601E+04 | 1.311E+04 | 1.380E+04 | 1.364E+04 |
| Galactinol | 4.577E+02 | 5.086E+02 | 2.640E+02 | 4.599E+02 | 4.056E+02 | 2.417E+02 |
| Nicotinate | 6.262E+03 | 1.566E+04 | 9.807E+03 | 7.187E+03 | 9.817E+03 | 6.953E+03 |
| Adenine | 6.947E+02 | 8.621E+02 | 1.756E+03 | 6.092E+02 | 5.919E+02 | 4.511E+02 |
| Ethanolamine | 7.077E+03 | 9.680E+03 | 1.300E+04 | 6.585E+03 | 7.225E+03 | 7.587E+03 |
| Putrescine | 1.506E+04 | 1.217E+04 | 6.999E+03 | 1.751E+04 | 1.109E+04 | 6.982E+03 |
| Guanidine | 1.779E+03 | 1.878E+03 | 2.414E+04 | 2.105E+04 | 5.487E+03 | 8.360E+03 |
| Uracil | 1.588E+02 | 4.941E+02 | 1.034E+02 | 3.952E+02 | 6.071E+02 | 3.088E+02 |
| 5,6-dihydrouracil* | 3.413E+05 | **4.417E+05** | **4.044E+05** | 3.538E+05 | **4.315E+05** | 3.910E+05 |
| Urea | 3.983E+03 | 3.982E+03 | 3.609E+03 | 4.162E+03 | 6.439E+03 | 6.214E+03 |
| Phosphorate | 5.730E+05 | 6.868E+05 | 7.055E+05 | 5.135E+05 | 5.751E+05 | 6.214E+05 |
| Palmitate | 6.829E+04 | 7.155E+04 | 9.085E+04 | 5.757E+04 | 6.487E+04 | 6.700E+04 |
| Pyruvate | 1.836E+03 | 2.924E+03 | 4.013E+03 | 1.691E+03 | 2.028E+03 | 1.966E+03 |
| Quinate* | **1.009E+06** | **1.019E+06** | 8.295E+05 | 8.413E+05 | 7.339E+05 | 5.831E+05 |
| Succinate | 7.806E+02 | 8.024E+02 | 5.673E+02 | 6.554E+02 | 6.896E+02 | 6.134E+02 |
| Threonate | 4.174E+03 | 5.019E+03 | 5.513E+03 | 2.494E+03 | 3.428E+03 | 4.006E+03 |
| 2 Methylmalate | 2.198E+02 | 2.682E+02 | 2.095E+02 | 1.790E+02 | 1.691E+02 | 1.234E+02 |
| 2 Oxyglutarate | 4.338E+02 | 8.187E+02 | 4.445E+02 | 3.912E+02 | 3.290E+02 | 2.718E+02 |
| 3,4-dihydroxybenzoate | 4.548E+02 | 8.643E+02 | 5.573E+02 | 5.926E+02 | 8.831E+02 | 7.558E+02 |
| 3CGA* | 8.575E+04 | **1.490E+05** | 8.046E+04 | 7.141E+04 | 7.123E+04 | 9.988E+04 |
| 4-hydroxy-benzoate | 1.173E+02 | 2.865E+02 | 2.175E+02 | 1.506E+02 | 2.809E+02 | 3.236E+02 |
| Benzoate | 6.296E+02 | 7.773E+02 | 6.234E+02 | 6.152E+02 | 8.921E+02 | 1.113E+03 |
| Glycerate | 3.504E+02 | 4.404E+02 | 6.032E+02 | 3.035E+02 | 4.187E+02 | 4.940E+02 |
| Fumarate | 4.516E+03 | 7.599E+03 | 5.496E+03 | 5.617E+03 | 8.733E+03 | 7.907E+03 |
| Glycolate | 3.254E+02 | 5.287E+02 | 4.883E+02 | 4.416E+02 | 5.033E+02 | 4.602E+02 |
| Isocitrate | 6.600E+04 | 9.121E+04 | 1.038E+05 | 3.946E+04 | 5.423E+04 | 7.501E+04 |
| Malate* | **4.486E+05** | **4.967E+05** | **4.646E+05** | 4.242E+05 | 4.542E+05 | 4.227E+05 |
| Octadecanoate | 7.554E+03 | 4.026E+03 | 8.330E+03 | 3.141E+03 | 3.468E+03 | 3.260E+03 |
| Citrate* | 1.526E+05 | 2.707E+05 | 2.088E+05 | 2.025E+05 | **3.688E+05** | **3.116E+05** |
| Dehydroascorbate | 2.517E+04 | 3.933E+04 | 5.374E+04 | 1.373E+04 | 2.109E+04 | 3.497E+04 |
| Erythronate | 1.454E+03 | 1.610E+03 | 1.701E+03 | 1.327E+03 | 1.543E+03 | 1.430E+03 |

Relative content calculated based on peak area of the analyte. Values are mean of 10 independent accessions. (**) shows highly significant metabolites and (*) significant. Highlighted values (**Bold**) across the rows show significantly higher values.

### 5.4.3 Metabolite profile of the African tomato fruit tissues

This section presents the metabolite profile of African tomato fruits in response to drought stress. The analysis by LC-MS allowed tentative identification and detection of 34 major metabolites as listed in Table 5.4. Most of the compounds reported in Table 5.4 have been detected previously in tomatoes enabled putative identifications with improved experimental data. However, quantification of these compounds could not be performed in these analyses, as most of the compounds detected are not commercially available as standards. Therefore, the intensities of the corresponding peaks were used to determine the relative abundance of the metabolites. The peak areas were taken to be directly proportional to the concentration and large peak areas indicated high relative abundance of primary metabolites and secondary metabolites. From the results, the main compound groups present in African tomato fruits were classified into flavonoids, phenolic acids and alkaloids. In addition, other miscellaneous metabolites, such as acyl sugars were annotated. From this study, the dominant flavonoids was observed to be quercetin and kaempferol derivatives whereas the major phenolic acids were chlorogenic acid, coumaric acid and caffeic acid derivatives. These compounds were identified as major fruit metabolites and exhibited variable levels in African tomato fruits.

Table 5.4: The metabolites and derivatives identified from African tomato accessions

| No | $R_t$. (min) | MW | Molecular formula | Maximum peak intensity [M+H]+ or [M−H]− | Identification Metabolite name |
|---|---|---|---|---|---|
| 1 | 7.83 | 191.0 | $C_6H_7O_7$ | 4.29E+05 | Citric acid |
| 2 | 10.42 | 164.1 | $C_9H_{11}NO_2$ | 3.39E+05 | Phenylalanine |
| 3 | 10.77 | 205.2 | $C_{11}H_{12}N_2O_2$ | 1.07E+05 | Tryptophan |
| 4 | 10.93 | 341.3 | $C_{15}H_{18}O_9$ | 9.88E+04 | Caffeoyl-glucoside |
| 5 | 11.26 | 355.2 | $C_{16}H_{19}O_9$ | 2.24E+04 | Feruloyl-glucoside |
| 6 | 11.28 | 353.4 | $C_{16}H_{18}O_9$ | 5.88E+05 | Caffeoyl quinic acid |
| 7 | 11.93 | 341.1 | $C_{15}H_{18}O_9$ | 2.32E+03 | Caffeic acid-hexose |
| 8 | 12.54 | 102.` | $C_4H_8NO_2$ | 1.46E+06 | GABA |
| 9 | 13.30 | 353.4 | $C_{16}H_{18}O_9$ | 7.87E+05 | 5-caffeoyl quinic acid |
| 10 | 13.91 | 355.1 | $C_{16}H_{20}O_9$ | 4.07E+06 | Ferulic acid-hexose |
| 11 | 13.93 | 179.0 | $C_9H_8O_4$ | 6.80E+06 | Caffeic acid |
| 12 | 14.10 | 1268.6 | $C_{58}H_{95}NO_{29}$ | 3.39E+06 | Esculeoside related |
| 13 | 14.44 | 741.4 | $C_{32}H_{37}O_{20}$ | 3.27E+08 | Q3RP |
| 14 | 14.55 | 353.1 | $C_{16}H_{18}O_9$ | 1.58E+06 | Chlorogenic acid |
| 15 | 15.25 | 725.4 | $C_{32}H_{37}O_{19}$ | 8.23E+06 | K3RP |
| 16 | 15.73 | 609.5 | $C_{27}H_{29}O_{16}$ | 4.67E+06 | Quercetin-3-rutinoside |
| 17 | 16.32 | 593.4 | $C_{27}H_{30}O_{15}$ | 2.25E+06 | K3R |
| 18 | 17.00 | 1048.5 | $C_{50}H_{83}NO_{22}$ | 3.21E+06 | Hydroxy-α-tomatine |
| 19 | 17.62 | 515.4 | $C_{25}H_{24}O_{12}$ | 3.57E+06 | Di-caffeoylquinic acid |
| 20 | 18.41 | 325.1 | $C_{15}H_{18}O_8$ | 1.38E+05 | Coumaric acid-hexose |
| 21 | 18.42 | 1032.0 | $C_{50}H_{83}NO_{21}$ | 6.89E+07 | Alpha-tomatine |
| 22 | 19.39 | 595.2 | $C_{27}H_{32}O_{15}$ | 5.34E+05 | Naringenin dihexose |
| 23 | 19.91 | 609.1 | $C_{27}H_{30}O_{16}$ | 5.07E+05 | Rutin |
| 24 | 20.14 | 425.2 | $C_{21}H_{30}O_9$ | 2.58E+06 | Abscisic acid-hexose |
| 25 | 20.86 | 433.1 | $C_{21}H_{22}O_{10}$ | 9.45E+05 | Naringenin hexose |
| 26 | 21.30 | 147.0 | $C_9H_8O_2$ | 8.54E+05 | Cinnamic acid |
| 27 | 24.88 | 264.3 | $C_{15}H_{20}O_4$ | 1.23E+07 | Abscisic acid |
| 28 | 25.24 | 291.0 | $C_9H_8O_3$ | 9.98E+05 | Coumaric acid |
| 29 | 25.82 | 299.1 | $C_{13}H_{16}O_8$ | 3.42E+06 | HBA-hexose |
| 30 | 26.10 | 1455.0 | –– | 2.32E+05 | Glycoalkaloids |
| 31 | 26.59 | 677.4 | $C_{34}H_{30}O_{15}$ | 3.32E+06 | Tri-caffeoylquinic acid |
| 32 | 27.18 | 271.2 | $C_{15}H_{12}O_5$ | 1.53E+06 | Naringenin |
| 33 | 26.10 | 562.3 | $C_{38}H_{43}O_{20}$ | 1.88E+05 | Acyl sugar |
| 34 | 27.78 | 271.2 | $C_{15}H_{12}O_5$ | 1.58E+07 | Naringenin chalcone |

Q3RP - Quercetin-3-rutinoside-pentoside; K3RP - Kaempferol-3-rutinoside-pentoside; K3R - Kaempferol-3-rutinoside; HBA-hexose - Hydroxybenzoic acid-hexose

### 5.4.3.1 Correlation of the LC-MS metabolites in African tomato fruits

In this study, the LC-MS dataset of the African tomato fruits was analyzed for correlations between metabolite intensity signals across the samples (Figure 5.18-21). The dataset comprised a total of 34 named metabolites and was used to assess the degree of linear association between

the metabolites where positive and negative correlation between the identified compounds was observed (Figure 5.18). Positive correlations were obtained for r > 0, indicating positive linear associations. Since majority of the metabolites showed positive correlation, the highest positive correlations were found for peak intensities belonging to hydroxybenzoic acid and 5-caffeoylquinic acid (r = 0.984), caffeic acid and 5-caffeoylquinic acid (r = 0.969), ferulic acid-hexose and GABA (r = 0.972); tri caffeoylquinic and 5-caffeoylquinic acid (r = 0.995); naringenin hexose and naringenin dihexose (r = 0.978); cinnamic acid and rutin (r = 0.977); tri caffeoylquinic acid and hydroxybenzoic acid (r = 0.981). On the other hand, negative correlations were obtained for r < 0, which indicated linear negative associations between two variables. The negative correlation was observed between α-tomatine and phenylalanine (r = -0.090), caffeoyl quinic acid (r = -0.047), 5-caffeoyl quinic (r = -0.072) and caffeic acid (r = -0.058). The cluster analysis of metabolite levels in different African tomato fruits at different treatments and ripening stages also showed clear correlation between the different treatments and ripening stages (Figure 5.13).

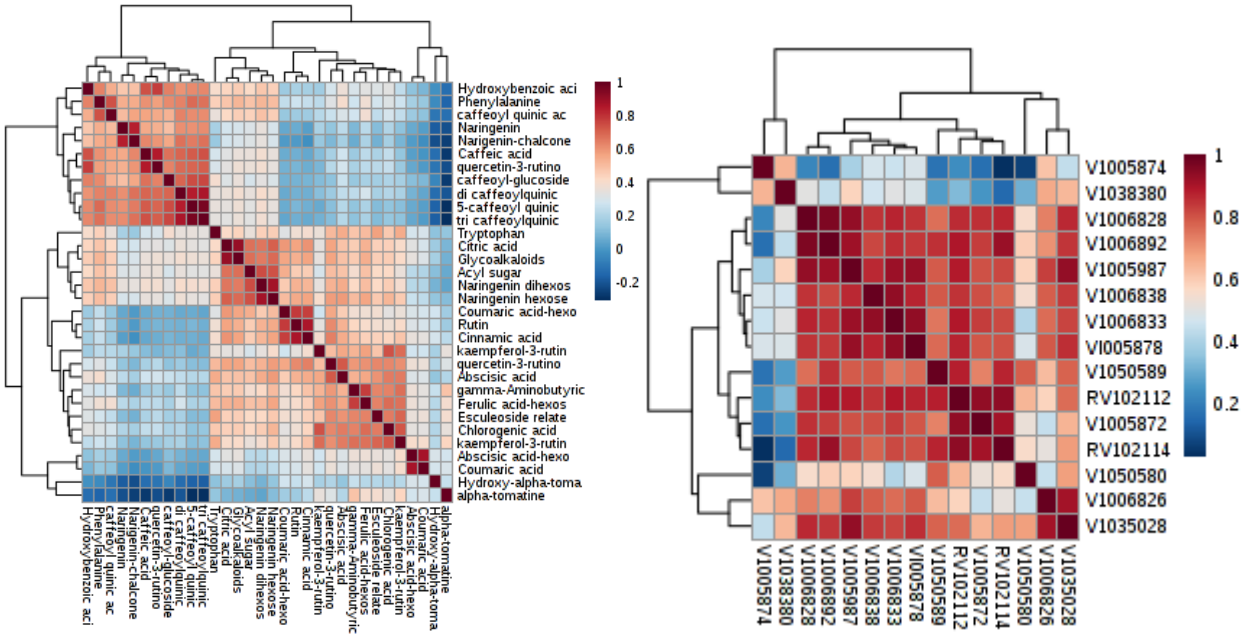



Figure 5.18: Pearson product-moment correlation analysis of African tomato fruit metabolites measured in ripening stages at different treatments. The colour key indicates the extent of correlation; red color indicates positive correlation and blue indicates negative correlation.

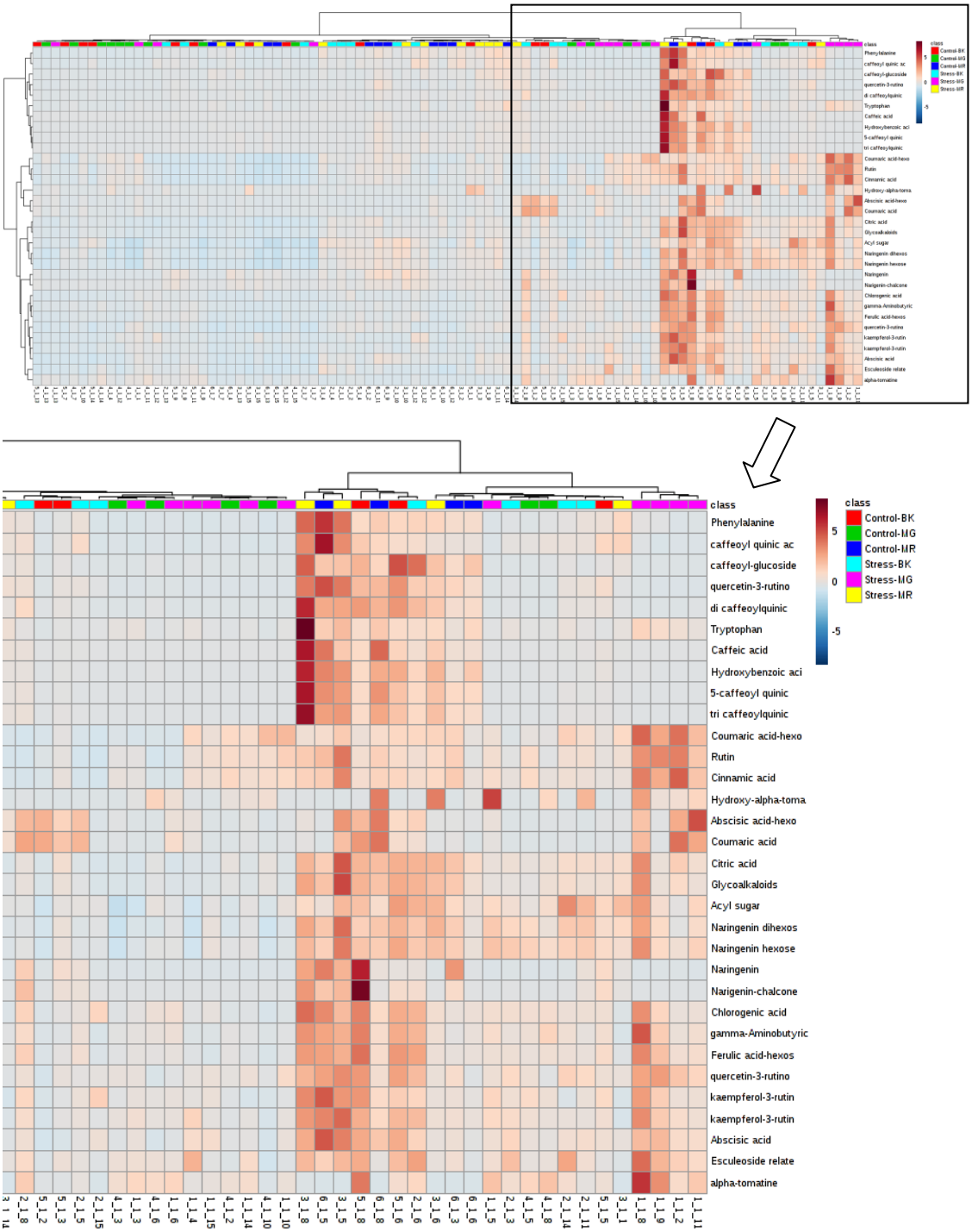



Figure 5.19: Heatmap representing the distribution of metabolites in individual African tomato fruits at different ripening stages under different conditions. The colors indicate the ripening stages: green (control mature green), red (control breaker), blue (control mature red), pink (drought stress mature green), light blue (drought stress breaker) and yellow (drought stress mature red). The colour key indicates the extent of changes in metabolites; red color indicates high accumulation and blue indicates low accumulation; (Appendix 2. for accession abbreviations)

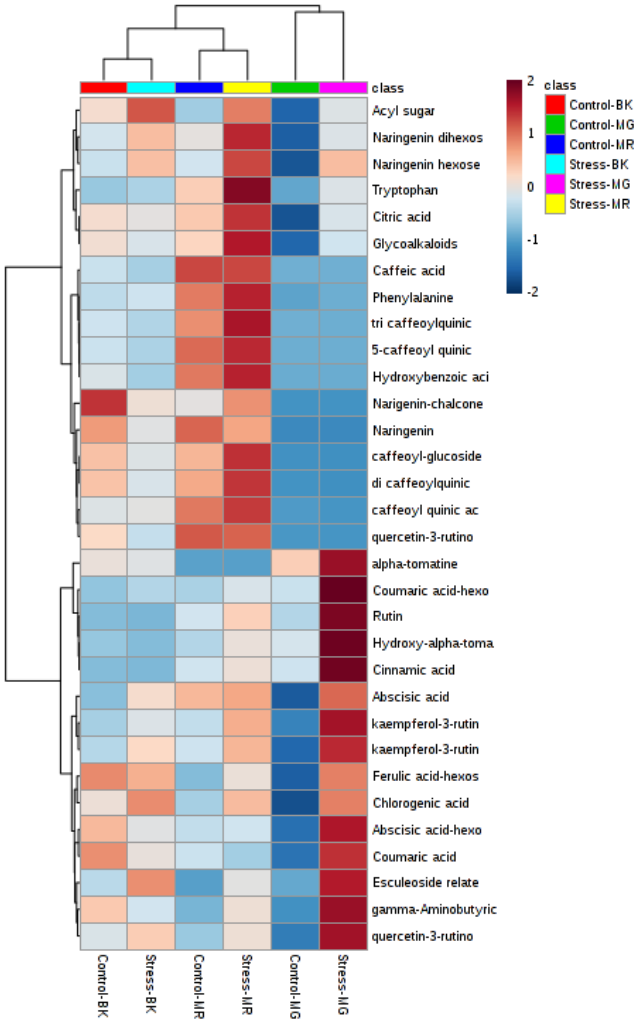



Figure 5.20: Heatmap showing correlation and cluster analysis of metabolite levels in different African tomato fruits at different treatments and ripening stages. The colors indicate the ripening stages: green (control mature green), red (control breaker), blue (control mature red), pink (drought stress mature green), light blue (drought stress breaker) and yellow (drought stress mature red). The colour key indicates the extent of changes in metabolites; red color indicates high accumulation and blue indicates low accumulation; (Appendix 2. for accession abbreviations)

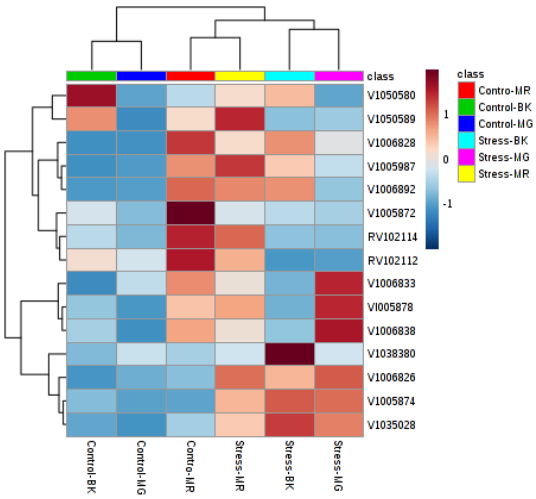



Figure 5.21: Heatmap representing the distribution of metabolites between the different African tomato fruits and ripening stages. The colors indicate the ripening stages: green (control mature green), red (control breaker), blue (control mature red), pink (drought stress mature green), light blue (drought stress breaker) and yellow (drought stress mature red). The colour key indicates the extent of changes in metabolites; red color indicates high accumulation and blue indicates low accumulation; (Appendix 2. for accession abbreviations)

The accessions with outlier characteristics were also determined **(**Figure 5.22). From the results accessions V1002112 (stress-mature green), V1005874 (stress-mature green), V1050580 (control-mature green), V1030380 (control-mature red) and V1035028 (control-mature red) were observed to be outliers.

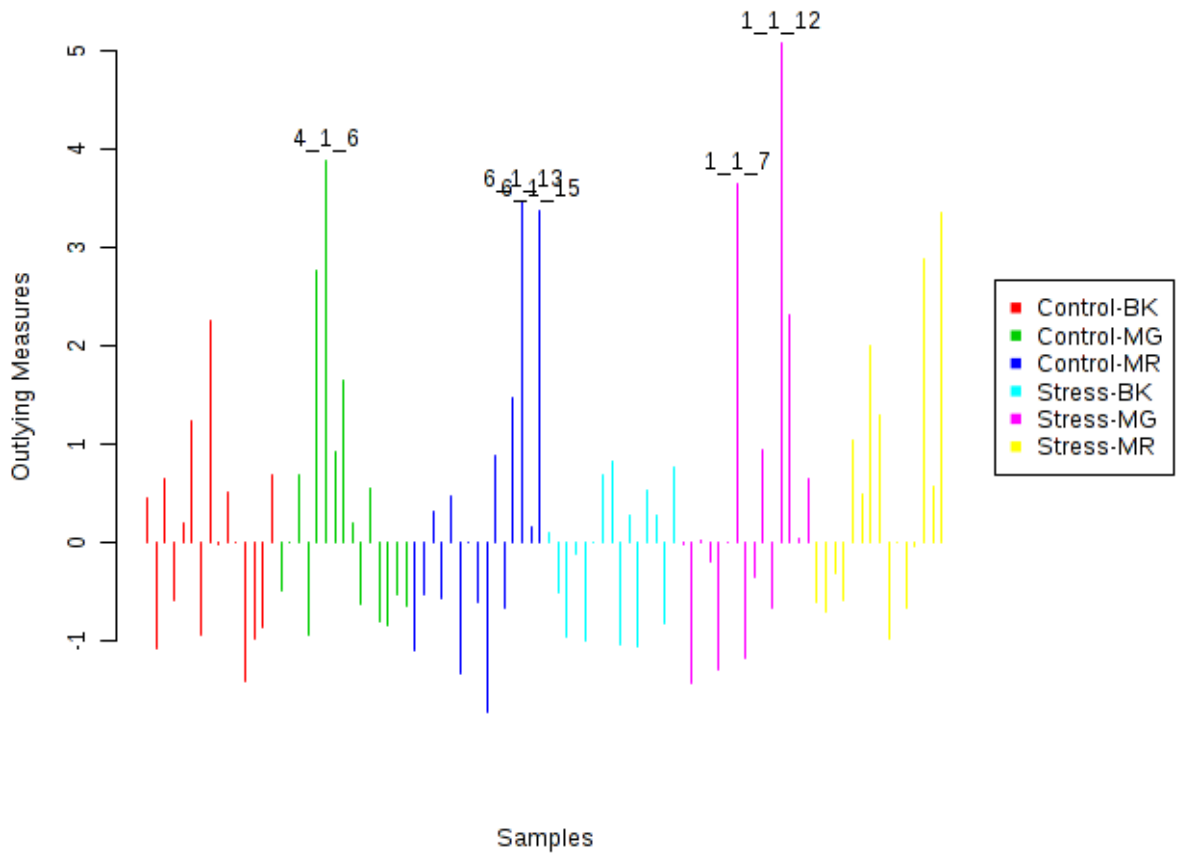



Figure 5.22: The top five potential outlier African eggplant accessions identified by Random Forest (Appendix 2(b). for accession abbreviations)

### 5.4.3.2 Hierarchical cluster analysis

In order to compare the LC-MS profiles of the different African tomato accessions, a PCA was performed using the entire data set of response peak intensities (Figure 5.23-25). These were visualized by plotting the scores of a PCA (Figure 5.23) to enable a distinction in the response between the three stages of ripening; mature green, breaker and mature red. From these profiles, most accessions were markedly separated from each other in the PCA plot. The plot showed differences between fruit samples according to their metabolite profiles based on metabolite-specific signal abundances. However, it appeared that metabolite differences between the different treatments and ripening stages were more pronounced than differences between accessions. In the PCA, the PC1 and PC2 explained 42.5 and 31.7 % of the variation in the metabolite profiles, respectively (Figure 5.24). Interestingly, a clear segregation between the control and drought stressed tomatoes was found where the largest metabolite changes were

observed between the two treatments, which corresponded to the first principal component in the PCA plot. The second components, in contrast, corresponded to fruit development as well as the different accessions. In addition, the samples were clustered mainly according to their ripening stages where the differences between fruits became more pronounced. This indicated an inter-accession and treatment variation in the metabolic profiles of fruits and supports a stronger ripening-driven variation than an accession-driven variation. This means that the metabolite profiles of the same ripening stages in different accessions are more similar than the profiles of the two different ripening stages within each accession. In addition, the PCA scores plot (Figure 5.24) showed the cherry tomatoes V1050580, V1050589, V1006838, V1005875 and V1002114 clearly separated from the other African tomatoes. From the PCA, it was also observed that quercetin-3-rutinoside-pentoside was the predominant metabolite in all the stages of ripening and treatments (Figure 25).

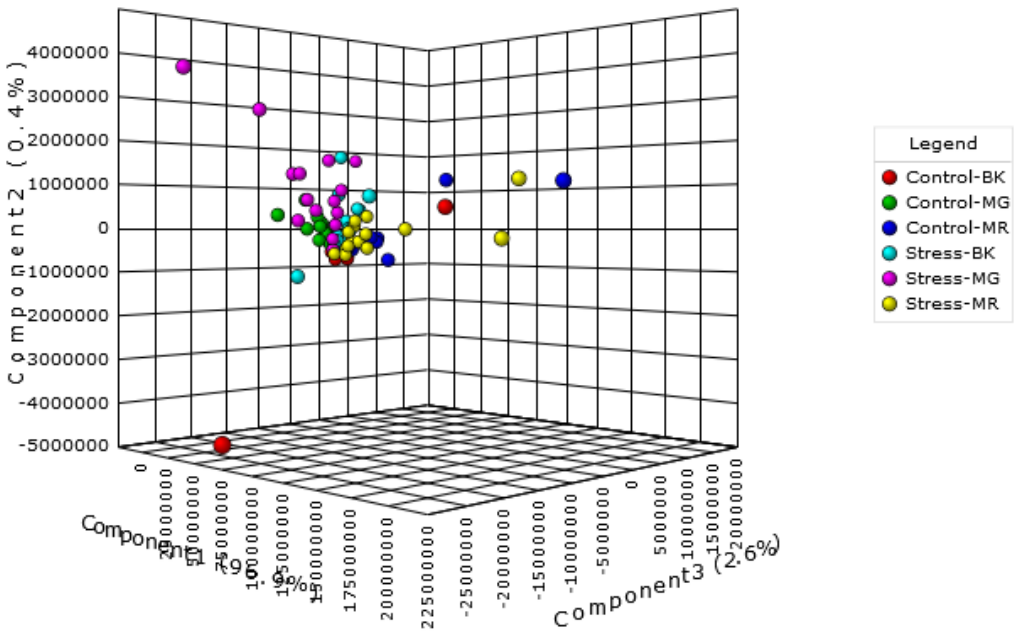



Figure 5.23: Three dimensional principal component analysis scores plot showing cluster of PC1, PC2 and PC3 of control and drought stressed African tomato fruits during different stages of ripening (Appendix 2. for accession abbreviations)

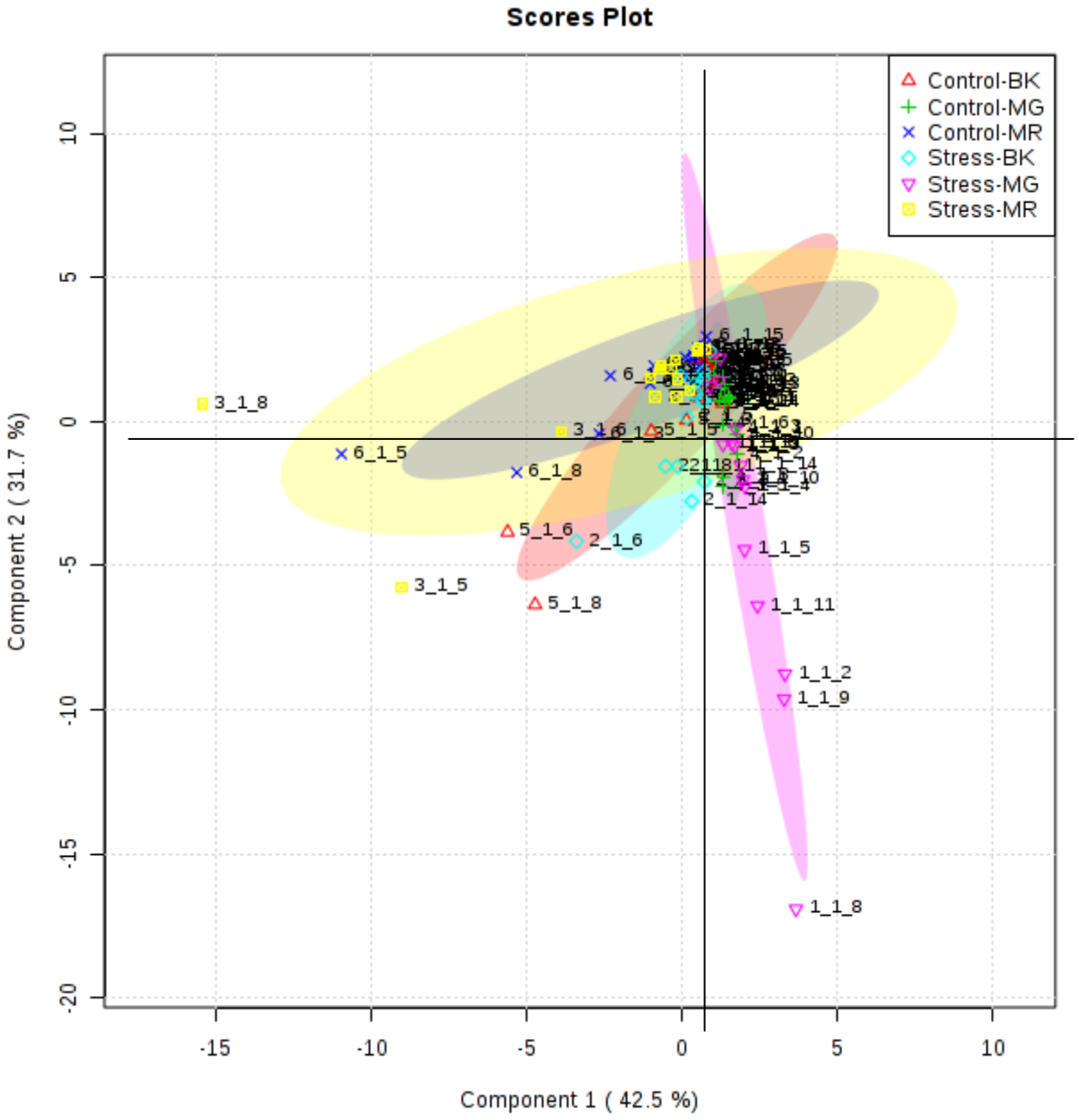



Figure 5.24: Principal component analysis showing PC1 and PC2 cluster of the control and drought stressed fruits of African tomato accessions at different ripening stages based on LC-MS metabolite data. (Appendix 2. for accession abbreviations)

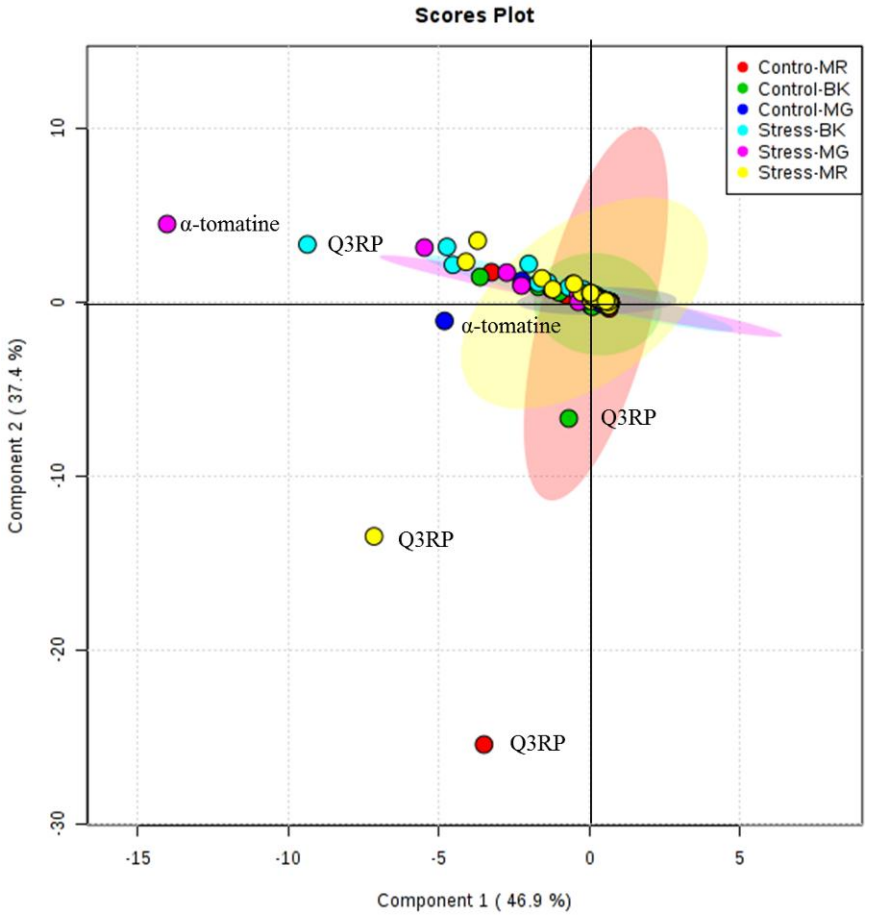



Figure 5.25: Principal component analysis scores scatter plot of metabolite profiles obtained from LC-MS in control and drought stressed African tomato fruits during different stages of ripening. The colors indicate the ripening stages: blue (control mature green), green (control breaker), red (control mature red), pink (drought stress mature green), light blue (drought stress breaker) and yellow (drought stress mature red). (Appendix 2. for accession abbreviations)

### 5.4.3.3 The profiles of secondary metabolites in African tomato fruits as influenced by drought stress

Metabolomic profiles in this study indicated that most of the important secondary metabolites were observed in the drought stressed accessions. It is interesting to note that the profiles obtained in the study obviously depended on the accessions examined, ripening stages and the treatment. Overall, the main flavonoids in many drought stressed accessions were predominantly quercetin and kaempferol derivatives as it showed variable levels in the fruit sample samples (Table 5.5). The specific metabolites identified were quercetin-3-rutinoside-pentoside, abscisic acid, α-tomatine, kaempferol-3-rutinoside-pentoside, narigenin-chalcone, quercetin-3-rutinoside, ferulic acid-hexose, esculeoside related and caffeic acid which were found to be significantly

high ($p < 0.05$) in the fruits. Similarly, GABA was found and was significantly high ($p < 0.05$) in mature green stage than breaker and mature red stages. However there was no significant difference ($p > 0.05$) between the GABA content of mature red and breaker stages. On the other hand, feruloyl-glucoside was not detected in the mature green stages of both control and stressed accessions. Caffeic acid-hexose on the other hand was present in the mature green and breaker stages and absent in the mature red stage of the fruits of stressed accessions but was absent in the fruits of the control accessions. Nevertheless, a number of the identified metabolites was seen to be affected by drought stress and was observed to increase in stressed accessions. Furthermore, the mature red fruits of the drought stressed crops were observed to be associated with an increase in most of the metabolites.

Table 5.5: Average relative responses of the African tomato fruit metabolites under control and drought stress conditions

| | Stress | | | Control | | |
|---|---|---|---|---|---|---|
| Metabolites | Mature green | Breaker | Mature red | Mature green | Breaker | Mature red |
| Citric acid | 5.32E+04 | 5.45E+04 | **7.20E+04** | 3.44E+04 | 5.67E+04 | 5.94E+04 |
| Phenylalanine | 4.88E+03 | 1.48E+04 | **4.27E+04** | 3.19E+03 | 1.32E+04 | 3.38E+04 |
| Tryptophan | 4.90E+03 | 3.46E+03 | 1.25E+04 | 1.70E+03 | 2.93E+03 | 6.64E+03 |
| Caffeoyl-glucoside | 4.92E+01 | 5.52E+03 | 1.35E+04 | 1.96E+02 | 8.31E+03 | 8.88E+03 |
| Feruloyl-glucoside | nd | 4.61E+02 | **4.28E+03** | nd | 2.31E+02 | 1.95E+03 |
| Caffeoyl quinic acid | 4.16E+03 | 2.36E+04 | **5.00E+04** | 5.04E+03 | 2.29E+04 | 4.25E+04 |
| Caffeic acid-hexose | 7.74E+01 | 6.83E+01 | nd | nd | nd | nd |
| GABA | 1.39E+05 | 9.33E+04 | **9.98E+04** | 7.18E+04 | 1.07E+05 | 7.92E+04 |
| 5-caffeoyl quinic acid | 3.73E+01 | 1.72E+04 | **9.87E+04** | 2.75E+02 | 2.60E+04 | 8.14E+04 |
| Ferulic acid-hexose * | 5.06E+05 | 4.69E+05 | 4.10E+05 | 2.28E+05 | 4.99E+05 | 3.23E+05 |
| Caffeic acid* | 1.40E+03 | 1.02E+05 | 6.38E+05 | 2.84E+03 | 1.78E+05 | 6.39E+05 |
| Esculeoside related* | 5.88E+05 | 4.81E+05 | **3.52E+05** | 2.24E+05 | 3.03E+05 | 2.12E+05 |
| Q3RP** | 3.73E+04 | 1.07E+07 | **2.95E+07** | 1.76E+05 | 1.77E+07 | **3.05E+07** |
| Chlorogenic acid | 1.91E+05 | 1.88E+05 | 1.74E+05 | 8.68E+04 | 1.57E+05 | 1.35E+05 |
| K3RP** | 1.25E+06 | **7.96E+05** | **9.77E+05** | 5.02E+05 | 6.90E+05 | 7.42E+05 |
| Quercetin-3-rutinoside* | 1.10E+06 | **8.63E+05** | **8.16E+05** | 5.63E+05 | 7.79E+05 | 6.93E+05 |
| Kaempferol-3-rutinoside | 2.24E+05 | 1.73E+05 | 1.85E+05 | 9.89E+04 | 1.46E+05 | 1.53E+05 |
| Hydroxy-α-tomatine | 2.97E+05 | 6.50E+04 | 1.31E+05 | 1.14E+05 | 7.44E+04 | 9.21E+04 |
| di caffeoylquinic acid | 2.00E+03 | 1.79E+05 | **4.52E+05** | 7.46E+03 | 2.81E+05 | 3.18E+05 |
| Coumaric acid-hexose | 3.23E+04 | 1.56E+04 | 1.74E+04 | 1.66E+04 | 1.39E+04 | 1.51E+04 |
| α-tomatine** | 8.86E+06 | 3.15E+06 | 2.33E+05 | 4.46E+06 | 3.49E+06 | 2.55E+05 |
| Naringenin dihexose | 9.55E+04 | 1.10E+05 | 1.35E+05 | 5.90E+04 | 9.35E+04 | 9.78E+04 |

| Metabolites | Stress | | | Control | | |
|---|---|---|---|---|---|---|
| | Mature green | Breaker | Mature red | Mature green | Breaker | Mature red |
| Rutin | 1.22E+05 | 5.41E+04 | **8.21E+04** | 6.35E+04 | 5.56E+04 | 6.91E+04 |
| Abscisic acid-hexose | 2.20E+05 | 1.10E+05 | **9.93E+04** | 1.64E+04 | 1.48E+05 | 9.30E+04 |
| Naringenin hexose | 1.68E+05 | 1.67E+05 | 1.91E+05 | 1.05E+05 | 1.46E+05 | 1.48E+05 |
| Cinnamic acid | 1.86E+05 | 7.78E+04 | **1.11E+05** | 9.93E+04 | 7.91E+04 | 9.94E+04 |
| Abscisic acid** | 1.81E+06 | 1.50E+06 | 1.66E+06 | 8.85E+05 | 1.20E+06 | 1.62E+06 |
| Coumaric acid | 9.76E+04 | 5.15E+04 | 3.32E+04 | 4.31E+03 | 7.77E+04 | 4.27E+04 |
| HBA-Hex | 7.37E+03 | 7.38E+04 | **4.53E+05** | 7.92E+03 | 1.50E+05 | 3.51E+05 |
| Glycoalkaloids | 2.45E+04 | 2.51E+04 | 3.85E+04 | 1.41E+04 | 2.70E+04 | 2.83E+04 |
| tri caffeoylquinic acid | 1.01E+02 | 7.81E+04 | 4.54E+05 | 1.64E+03 | 1.16E+05 | 3.09E+05 |
| Naringenin | 8.99E+02 | 7.87E+04 | 1.30E+05 | 5.85E+02 | 1.35E+05 | 1.60E+05 |
| Acyl sugar | 3.25E+04 | 4.33E+04 | 4.11E+04 | 2.01E+04 | 3.44E+04 | 2.86E+04 |
| Narigenin-chalcone* | 3.27E+02 | 6.70E+05 | 1.08E+06 | 4.88E+03 | 1.41E+06 | 6.09E+05 |

Relative content calculated based on peak area of the analyte. Values are mean of 15 independent African tomato accessions. (**) shows highly significant metabolites and (*) significant. Highlighted values (**Bold**) across the rows show significantly higher values. Q3RP - Quercetin-3-rutinoside-pentoside; K3RP - Kaempferol-3-rutinoside-pentoside; HBA-Hex - Hydroxybenzoic acid-hexose.
nd - not detected

## 5.5 DISCUSSION

This study provides an overview of drought stress acclimatization in African eggplant leaves and fruits and tomato fruits highlighting a number of metabolic processes. From the results, significant metabolite changes were observed on the leaves and fruits of the stressed accessions. The levels of the individual sugars varied considerably among the accessions, within the treatment, growth and ripening stages. For the African eggplant leaf tissues, an increase in most sugars was observed at the advanced stage (week 4 of growth). Other studies have reported that older leaves accumulate higher amounts of metabolites including sugars (Chaves *et al.*, 2009). The sugars in leaf tissues were also affected by drought stress. The results of this study concur with the findings by Sicher *et al.* (2012) which reported accumulation of a variety of sugars such as sucrose, raffinose, fructose, trehalose and maltose and decreased levels of myo-inositol in water-stressed barley. This likely represents adaptive response that helps plants cope with drought stress via osmotic and non-osmotic mechanisms. These soluble sugars are important components in plant metabolism as a source of carbon and energy within a cell and since they are related to photosynthesis, their level may be affected by different stresses (Bowne *et al.*, 2012; Zhao *et al.*, 2013). They act as osmolytes which function as osmoprotectants during water deficit (Bowne *et al.*, 2012). They also act as signaling molecules (Hanson and Smeekens, 2009)

and play important role in drought tolerance in plants by reducing the detrimental effects of osmotic stress, maintenance of turgor, stabilizing cell membranes and protecting plants from damage (Basu *et al.*, 2007; Bowne *et al.*, 2012). On the other hand, changes in xylose, a key component of cell walls, suggests that an additional way the African eggplants may cope with drought stress is through cell wall modification, as has been observed in other species (Warren *et al.*, 2012). Moreover, trehalose has been shown to be related to stress tolerance in bacteria and fungi (Rodziewicz *et al.*, 2014), and in the present study, it accumulated in the stressed accessions than the control accessions, therefore may play a protective role during abiotic stress. Although significant increase was observed in the levels of sucrose, fructose, trehalose, mannose and xylose with drought stress, glucose levels decreased in leaves of the drought stressed accessions. Glucose is a product of photosynthesis in plants, a process dependent on water. Since drought stress is characterized by low water level, it confers negative impact on the synthesis of glucose and other sugars (Massacci *et al.*, 2008; Chaves *et al.*, 2009). Reduction of photosynthesis arises due to the affected photosynthetic pigments, particularly chlorophylls which are responsible for the synthesis of sugars by plants and adversely affected during stress (Lizana *et al.*, 2006). From the study, reduced chlorophyll level was observed in the African eggplants subjected to drought stress and this may explain partly the decreased glucose levels under drought stress. Besides the alterations in photosynthesis and cell growth, drought stress has also been reported to affect the activities of Calvin cycle enzymes. This disrupts the carbohydrate metabolism thus decreased levels of glucose in leaves. Whereas most of the accessions reported a significant decrease ($p < 0.05$) in glucose levels during stress at weeks 2 as compared to controls, the following accessions RV100343, RV100199, RV100201, RV100273 and RV100246 demonstrated increased levels. These accessions showed strong correlations between the accumulation of several sugars (sucrose, glucose, fructose and trehalose) and drought tolerance. This was evident since the accessions were more resistant to drought as observed by delayed wilting compared to the other accessions. Interestingly, this study also revealed higher levels of glucose and fructose than sucrose. This is most probably due to the high invertase activity present in the plant leaves which has been observed in tomato. Invertase cleaves sucrose into hexoses (mainly glucose and fructose) to provide cells with fuel for respiration and with carbon and energy for the synthesis of numerous different compounds (Gibeaut *et al.*, 1990).

Drought stress was shown to affect the amino acid metabolism in plants, since significantly high ($p < 0.05$) levels were observed for the stressed fruits as compared to the control.

The present study revealed that the accessions which indicated higher stress susceptibility such as RV100273, RV100432, RV100327 and RV100445 had increased amino acid content in leaves and fruits as compared with the more drought-resistant accessions. The accumulation of proline by the sensitive phenotype has also been observed in other solanaceae crops such as Andean potato (Schafleitner *et al.,* 2007). GABA has also been suggested to be involved in cellular signaling in plant response to drought stress (Sarvajeet and Narendra, 2010). Therefore an increase in GABA may be responsible for triggering defense mechanism in fruits of drought-stressed plants. This was observed for the African solanaceae leaves and fruits thus may reflect their stress tolerance. The results of this study agree with findings of other studies which have reported accumulation of amino acids particularly proline and GABA in many plant species in response to drought stress (Shtereva *et al.*, 2008; Witt *et al.*, 2012; Hanci and Cebeci, 2014). Proline is one of the most widely distributed osmolyte and an ROS scavenger synthesized in response to drought stress (Szabados and Savoure, 2010) and confers protection against oxidative damage to plants (Rodziewicz *et al.,* 2014). On the other hand, glutamate is precursor of many stress-related compounds and a higher pool of glutamate was hypothesized to lead to faster production of defense metabolites (Figueiredo *et al.*, 2008). Accumulation of glutamate is known to regulate and integrate the metabolism in stressed photosynthetic tissues (Chaves *et al.*, 2009). This may explain increased glutamate in the African eggplants in response to drought stress as reported in this study. On the other hand, there was dramatic drop in aspartate and this indicates that, the conversion of oxaloacetate to aspartate by aspartate aminotransferase may have been inhibited under drought stress, resulting in its decrease (Rabara *et al.*, 2015). Similarly, pyruvate metabolism was also inhibited, causing a reduction of alanine and isoleucine. Glycine and alanine were found to be more abundant in the leaves of control plants compared with the drought stressed plants. Contrary to this, the drought stressed fruits had increased glycine as compared to control. This concur with the findings by Meyerowitz *et al.* (2012) which proposed that the accumulation of glycine in transgenic plants can enhance drought tolerance because it is a source of precursors for key metabolic pathways. Furthermore the branched amino

acid, isoleucine also displayed high levels throughout plant growth and this have a role in respiration as alternative substrate under stress (Kochevenko *et al.*, 2012).

Organic acids are important components in plants and strongly influence their taste and overall quality. The accumulation of most of the organic acids after drought treatment might be connected with the demand for these compounds as substrates for secondary metabolite pathways connected with the plant defense. The obvious role of quinic acid is as a precursor for phenolic compounds and other secondary metabolites (Liu *et al.*, 2009). Therefore, differences in amounts of quinic acid could also be related to metabolism or synthesis of secondary metabolites. Most of the organic acids increased following drought stress and interestingly, fumaric acid and malic acid levels seem to correlate positively with developmental stages and stress. They had quite different responses, probably resulting from the different functions performed by these components in the species. These acids have been demonstrated as carbon sources related to plant growth (Fernie and Martinoia, 2009). Malate plays an important role as photosynthetic intermediate, an essential storage carbon molecule and as intermediate of the TCA cycle in all plant species (Fernie and Martinoia, 2009). In addition, several recent evidences suggest that fumarate and malate play an important function as pH regulator and exhibits partial control over the efficacy of nutrient uptake and over stomatal function (Fernie and Martinoia, 2009). Previous studies found similar responses with most organic acids and TCA-cycle intermediates reporting an increase in response to drought stress in different plant species (Urano *et al.*, 2009). Contrary to these are the findings of a study on wheat cultivars which reported that most organic acids decreased following drought stress (Bowne *et al.*, 2012). Although the TCA pathway in plant is well known, its regulation is still poorly understood (Fernie *et al.*, 2004).

For the African tomato fruits, the results demonstrated high levels of different classes of flavonoids (quercetin, kaempferol and naringenin), hydroxycinnamic acids (caffeic, chlorogenic, ferulic and p-coumaric acids) and glycoalkaloid compounds. In a similar vein, flavonoid derivatives were found to be the major metabolites in the fruit tissues. From the results, a well-established metabolite variation was observed for the different fruit morphologies as well as the different ripening stages. It was also found out that the levels of majority of metabolites were considerably higher in accessions under water deficit. In line with this study, previous studies

have also reported quercetin and their derivatives as the most common and abundant flavonol present in tomato fruits (Le Gall *et al.*, 2003; Slimestad and Verheul, 2009). The results of the study also concur with previous studies investigating the phenolic composition of tomatoes, where flavonols, hydrolysable and condensed tannins and hydroxycinnamic acids (such as chlorogenic acids) emerged in the fruits of tomatoes (Dumas *et al.*, 2003; Moco *et al.*, 2006). Similarly the obtained metabolic profiles were comparable to the ones previously described and published in LC-MS database of tomato fruit metabolites (Moco *et al.*, 2007). In addition, accumulation of such metabolites has been observed to occur in plants subjected to stresses and was expected to be found in fruit. This is because they are important factors in the reproduction, development and defense mechanisms of plants therefore play a major role in the adaptation of plants to the environment and in overcoming stress conditions (Araujo and Telhado, 2015). Beside, these metabolites are also important for the functional and sensory properties (such as taste, colour and nutrient qualities) of fruits and fruit-based products (Dumas *et al.*, 2003). Since these metabolites are widely distributed in plants, it indicates that they are abundant in the human diet (Calderón-Montaño *et al.*, 2011). Besides their importance in plants, quercetin and kaempferol displays relatively high antioxidative potential owing to its phenolic hydroxyl groups (Dumas *et al.*, 2003). Epidemiological studies have implied that high intake of these metabolites reduces the incidence of some types of cancer and cardiovascular diseases. Owing to his, it would appear that African tomato fruits are promising crops for bioactive/functional flavonol-rich ingredients.

## 5.6 CONCLUSION

This study presents a clear discrimination in the levels of metabolites in response to drought stress and this was dependent on accession, stage of growth and ripening. This provides evidence of drought and oxidative stress responses in African eggplant and tomato accessions, as indicated by increased levels of compatible solutes such as sugars and amino acids as well as antioxidants. Majority of the metabolites showed progressive accumulation towards the last stage of ripening therefore indicates that consumption of ripe fruits is important. Some of the observed metabolite compositional changes are related to known phenomena associated with development, stress and photosynthetic activity. Besides, this study also demonstrated that many miscellaneous metabolites can be produced under drought stress. These include seven (7) important metabolites

(proline, glutamate, sucrose, fructose, glucose, trehalose and citric acid) which accumulated during stress. The study provides evidence that proline, glutamate, sucrose, fructose, trehalose and citric acid are positively associated with stress tolerance in African eggplant, African tomato and possibly other plants. Together with these metabolites, secondary metabolites such as flavonoids, alkaloids and phenolic compounds were also observed in the African tomato fruits. These compounds are important due to their antioxidant activity and play an important in enhancing drought tolerance in these crops. The findings of this study, illustrates the common effects associated with drought stress on vegetable quality and this involves metabolite composition. This work confirms that African eggplants and tomatoes are potentially important crops in adapting to drought stress effects. Besides these metabolites being essential components in plant stress tolerance, the observed metabolites together with antioxidant compounds such as flavonoids and phenols may also contribute to the nutritional value and medicinal value of the leaves and fruits. In conclusion, African eggplants and tomatoes are a good source of nutritional and biologically active substances; therefore, they are promising nutraceutical and functional foods. Moreover, the study also provides information that may, with further experimentation, allow elucidation of the regulation of biochemical pathways underlying stress tolerance in these cops.

# CHAPTER SIX

# *IN VIVO* ANTIDIABETIC ACTIVITY OF THE AQUEOUS FRUIT EXTRACT OF SOME AFRICAN EGGPLANT FRUITS IN ALLOXAN INDUCED DIABETIC MICE

## 6.1 ABSTRACT

African eggplant fruits have been used by some African countries to manage diabetes mellitus. However, little is known concerning their therapeutic activity as well as their safety. The study aimed at investigating the antidiabetic activities of the fruit extracts of five selected African eggplant accessions. Male BALB/c mice were used in the study and were randomly grouped into four groups of five mice each. Freshly prepared 10% alloxan monohydrate dissolved in normal physiological water was used to induce diabetes in the mice. The extracts (100 and 300 mg/kg bwt) and metformin drug (30 mg/kg btw) were administered to the animals orally using a gavage on a daily basis for 28 days. Body weight, blood glucose concentrations and biochemical parameters; alanine aminotransferase (ALT), bilirubin, hemoglobin, triglycerides and urea were determined. The body weight of the diabetic mice decreased whereas the blood glucose increased immediately after induction of diabetes. Upon administration of the extracts and metformin, a gradual increase in body weight and decrease in blood glucose was observed. Significant glucose reduction was observed with 300mg/kg than 100mg/kg extracts. Metformin treated mice recorded the biggest fall in blood glucose levels (48.08 %) followed by RV10333 (44.86 %), RV10511 (41.66 %), RV10265 (40.01 %), RV101201 (35.23 %) and RV10445 (17.23 %). There was no significant difference ($p > 0.05$) between the bilirubin and ALT levels of all the extract and metformin treated mice and non-diabetic mice. The hemoglobin level of all the groups of mice including the untreated diabetic mice was within the normal level 8 – 12 mg/kg. Untreated mice reported significantly high ($p < 0.05$) levels of bilirubin 20.63±3.40 triglycerides (4.59±0.33), ALT (82.20±7.59) and urea (19.81±2.28). The results of this study therefore indicate that eggplant extracts possess antidiabetic properties, thus could be used in management of diabetes mellitus.

## 6.2 INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder associated with huge social, health, and economic consequences in many countries (Shabeer *et al.*, 2009). It is known to increase low-density cholesterol and decrease high-density cholesterol thus triggering coronary diseases (Rangika *et al.*, 2015). It is considered a major global burden (International Diabetes Federation, 2014) with 347 million people worldwide suffering from the disease. According to World health organization (WHO, 2013), 1.5 million deaths were reported to be directly caused by diabetes in 2012. The disease also causes more than 80 % of deaths in the low and middle-income countries (American Diabetes Association, 2013). In Kenya, over two million people are reported to have diabetes, with more susceptibility to urban people due to the erosion of traditional living and eating habits (Division of non-communicable diseases, 2010). Generally, diabetes is characterized by the sustained elevation of blood glucose concentration which greatly exceeds the normal upper limit leading to hyperglycemic condition (Kumar *et al.*, 2011b). The main aim in the treatment and management of the disease is to maintain blood glucose levels as near to normal as possible, while avoiding hypoglycemia (Kaul *et al.*, 2013). To achieve this, various strategies involving education exercise/activity, diet, oral medications (use of synthetic drugs) and/or insulin are often used in combination (Trivedi *et al.*, 2004). The use of drugs should not only correct the sugar levels in the blood but also prevent the development of other complications of diabetes. Unfortunately, current treatment for diabetes with synthetic hypoglycemic agents has been shown to cause adverse effects including hypoglycemia, gastrointestinal disturbances, renal toxicity and hepatotoxicity (Caprio and Fonseca, 2014). There is need therefore to look for safe and effective treatment and management strategies for diabetes.

Besides synthetic drugs, diet also plays a major role in the management of diabetes mellitus (Keter and Mutiso, 2012). According to World Health Organization (WHO), up to 80 % of the world's population in developing countries relies on traditional medicine practices for their primary health care needs (Musila *et al.*, 2002). This involves the use of plants to treat many diseases. Plants are known to be a valuable source of chemicals used for health and food industry (Rates, 2001; Balasundram et al., 2006). They contain a great diversity as well as a remarkably diverse array of bioactive compounds and secondary metabolites which makes them a possible

source for different types of drugs (Balasundram et al., 2006). The use of various plant remedies, formulations or herbal drugs has been reported to offer a suitable alternative treatment and management for diabetes mellitus. This has been documented and practiced in Ayurvedic and traditional medicine by different cultures around the world (Wang *et al.*, 2013). In addition, there is epidemiological evidence that demonstrates the protective role of diets rich in fruits and vegetables on diabetes (Widmer *et al.*, 2014). African eggplant fruits have been widely consumed in many African countries and their fruits can be sweet or bitter in taste. The bitter cultivars have been used as medicine (Chadha and Mndiga, 2007) for the treatment of various ailments due to its therapeutic activity. In this vein, epidemiological studies have shown that consumption of raw eggplant fruits is associated with a reduced risk of cancer and cardiovascular diseases (Clinton, 1998; Giovannucci *et al.*, 2002). Besides, there is also increasing evidence that the intake of their leaves and fruits have favorable impact on the incidence of many chronic diseases including diabetes (Kwon *et al.*, 2008). This protective effect may mainly be attributed to its valuable bioactive components with antioxidant properties (Borguini and Torres, 2009). The bioactive components are of health or nutraceutical significance therefore, authenticates the usefulness of these crops for medicinal purposes (Briskin, 2000). These reports that African eggplants have bioactivity against several diseases including diabetes mellitus have not been scientifically evaluated. In addition, little is known concerning antidiabetic activity of the extract as well as its underlying mechanisms and safety. Hence, the primary objective of the study was to investigate the in vivo hypoglycemic activity of aqueous fruit extracts of African eggplant in alloxan induced diabetic BALBc mice. Alloxan is a naturally occurring, broad spectrum antibiotic and cytotoxic glucose analogue. It is particularly toxic to the pancreatic, insulin producing beta cells in mammals and causes diabetes by inhibiting the glucose sensor of the β cell hence preventing glucose induced insulin secretion (Ashish and Swapnil, 2011). This study therefore contributes additional knowledge to the use of African eggplant in the management of diabetes

## 6.3 MATERIALS AND METHODS

### 6.3.1 Study design

Five African eggplant accessions; RV10265, RV10445, RV10333, RV10511 and RV101201 were used in the study. They were grown in a greenhouse at the Jomo Kenyatta University of

Agriculture and Technology (JKUAT), Kenya during January – July 2016 under carefully controlled and optimal growth conditions (12 h light/12 h dark conditions; room temperature, forest soil). The eggplant seeds were germinated in trays and the seedlings transplanted after four weeks of germination and grown alongside each other in 15 cm-diameter pots using forest soil and farm yard manure in the ratio 3:1. The spacing of 30 cm between plants and 50 cm between the rows was maintained. Irrigation was maintained throughout the experiment and fruits were collected at mature red stage. The antidiabetic study was undertaken at the Department of Biochemistry, College of Health Science, JKUAT, from July to December 2016.

### 6.3.2 Sample preparation

Fruits of five selected African eggplant accessions were used in this study to evaluate the antidiabetic effect on alloxan induced diabetic mice. Fresh mature red fruits were harvested and chopped into small pieces while removing the seeds and freeze dried using Alpha 1-4 LD Plus freeze drier (Christ, Germany). The dried tissues were then ground into powder using a mechanical grinder and to avoid any deterioration, they were kept in the cold room at -20 $^{0}$C until further use. All the reagents used in the study were purchased as shown in Appendix 1.

### 6.3.3 Preparation of aqueous extracts of African eggplant

Two hundred grams (200 g) of each of the powdered freeze dried African eggplant fruits were weighed and soaked separately in 1000 ml of distilled water. The contents were warmed in a water bath for 6 hours at 60 $^{0}$C, then left to cool at room temperature and then decanted into dry clean conical flask through folded cotton gauze stuffed into a funnel. The decanted extract was then filtered using filter papers under vacuum pump. The filtrate was then freeze-dried to powder for 72 hours. The freeze-dried powder was then stored in airtight container at -20 °C until used for the antidiabetic analysis. The percentage extract yield was determined according to the following equation for all the extractions.

$$\text{Extract yield (\%)} = \frac{\text{Weight of extract}}{\text{Weight of freeze dried sample}} \times 100$$

### 6.3.4 Hypoglycemic activity screening

BALBc mice 4-6 weeks old (25-40 g) were used in the study. These animals were purchased from Institute for Primate Research, Kenya and were maintained at JKUAT. They were housed at Biochemistry department, JKUAT animal house under standard environmental conditions of temperature at 25 + 2 °C under a 12 h dark-light cycle, and allowed free access to drinking water and standard pellet diet (mice cubes). After 1 week acclimatization period, the animals were randomly housed in thirteen cages with five mice per cage and experiments conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals. The experimental protocols and procedures used in this study were approved by the JKUAT Ethics Committee for the Care and Use of Laboratory Animals.

### 6.3.5 Experimental design

The experimental mice were randomly divided into four groups of five mice each prior to experiment. Group I consisted of alloxan induced diabetic mice orally administered with 100 and 300 mg/kg body weight (bwt) of African eggplant aqueous fruit extracts daily for 28 days; Group II consisted of alloxan induced diabetic mice (treatment diabetic group) orally administered with Glucophage (metformin) antidiabetic conventional drug daily for 28 days (30 mg/kg bwt); Group III consisted of alloxan induced diabetic mice without any treatment; Group IV consisted of normal non-diabetic mice (negative control). Group I experiment was further subdivided into five sub groups depending on the number of fruits (five in this case) namely; RV10265, RV10445, RV10333, RV10511 and RV101201. Each sub group was further subdivided into two of five mice each depending on the concentrations of the extracts (100 and 300 mg/kg bwt). The extracts and the metformin drug were administered to the animals orally using a gavage on a daily basis for 28 days. The treatments of all groups were carried out at the same time (in the morning) and under the same conditions. All the mice received normal diet (mice pellets) and distilled water.

### 6.3.6 Induction of diabetes and blood glucose tests

Freshly prepared 10% alloxan monohydrate dissolved in normal physiological water was used to induce diabetes in the mice. The mice were injected intraperitoneally with 150.0 mg/kg bwt of the prepared alloxan and allowed to feed on normal mice pellets for 48 hours. Forty-eight (48)

hours after alloxan administration, blood glucose level was measured using a glucometer. Severity of the induced diabetic state was assessed immediately before alloxan injection and by daily monitoring of blood glucose levels. Mice with blood glucose levels above 12 mg/dl after alloxan were considered diabetic and used in this study. Prior to initiation of this experiment, the animals were fasted for 8-12 hours (Szkudelski, 2001) but allowed free access to water until the end of this experiment. The extract and metformin were orally administered to diabetic mice at different doses of 100 mg/kg and 300 mg/kg bwt (extract) and 30 mg/kg bwt (metformin).

### 6.3.7 Blood sampling, blood glucose determination

Body weight and blood glucose level was measured and recorded before pretreatment and every 3 days after commencement of treatment. Growth performance and body weight were also assessed throughout the experimental period as described by Kumar *et al.* (2012). Blood sampling was done by sterilizing the tail with 10% alcohol and then nipping the tail at the start of the experiment. Bleeding was enhanced by gently "milking" the tail from the body towards the tip. After the operation, the tips of the tail were sterilized by swabbing with 70% ethanol. The blood glucose levels were determined with a SD Codefree blood glucose monitoring system (SD Biosensor, Inc., Republic of Korea).

### 6.3.8 Biochemical analysis

After 28 days, the animals were fasted overnight and were killed by inhalation of a high concentration of $CO_2$. Blood samples were then collected by cardiac puncture and used for biochemical analysis. These assays were performed as previously described (Kim and Park, 2015). Plasma was separated immediately after blood sampling by centrifugation at 10000×g for 10 min. The biochemical parameters analyzed involved liver, kidney and pancreatic parameters such as alanine aminotransferase (ALT), bilirubin, hemoglobin, triglycerides and urea. Plasma concentration of these parameters were analyzed using reflontron biochemical analyzer using their respective strips or assay kits (Roche Diagnostics, Mannheim, Germany) and standards; reflontron precinorm U (for bilirubin, triglyceride) reflontron precinorm HB (for hemoglobin). In addition, the internal organs such as pancreas, kidney and liver were kept to be used for toxicity test.

### 6.3.9 Data management and statistical analysis

The Data was entered in the Microsoft Excel Spread Sheet, cleaned and then exported to SAS statistical software version 9.1.3 for analysis. Results were expressed as mean ± SD of the number of animals used per every study point. Statistical analysis were done using ANOVA and post-ANOVA to compare the means of untreated normal control mice with diabetic mice treated with the conventional drug, and diabetic mice treated with plant extract at different doses. The values of $p \leq 0.05$ were considered to be significant.

## 6.4 RESULTS

The freeze dried powder of the African eggplant fruits gave a yield of 9.67±1.58 % w/w aqueous extract. The diabetic mice treated with extracts were compared with three controls; normal un-diabetic, diabetic untreated and diabetic treated with the conventional antidiabetic drug metformin. Significant variation was observed in the different extracts for the different treatments.

### 6.4.1 Effects of African eggplant fruit extracts on body weight

The results of the study (Table 6.1) indicate that mice in all the diabetic treatments (Group I – III) except the non-diabetic treatment (Group IV) had a decreased loss of body weight immediately after induction of diabetes. The decrease was observed in the diabetic mice as compared to the normal control mice which maintained a slight increase in weight. However, upon continuous administration of the extracts and treatment with the standard drug, a gradual increase in weight was observed from the $6^{th}$ day. Although the mice in metformin, RV10511 and RV101201 extract treatment gained more weight than the untreated mice, the weight increase for the mice treated with the two extracts was slightly less than for the metformin treated group. There was no significant difference ($p > 0.05$) in the weight increase among groups treated with RV10333, RV10265 and RV10445 extracts.

Table 6.1: Mean body weights (g) of alloxan-induced diabetic male BALB/c mice treated with African eggplant extract and metformin over the treatment period

| **Group** | | **Conc mg/Kg** | **Days after treatment** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | **0** | **2** | **6** | **10** | **14** | **18** | **22** |
| Group I | RV10265 | 100 | 27.0±3.5 | 26.3±3.0 | 24.84±1.7 | 26.5±3.3 | 27.8±2.4 | 28.1±2.7 | 29.2±3.1 |
| | | 300 | 23.8±2.2 | 23.2±1.9 | 21.92±2.3 | 24.7±2.0 | 25.78±2.6 | 26.3±2.4 | 28.1±1.9 |
| | RV10445 | 100 | 23.7±3.1 | 23.0±2.5 | 21.69±2.4 | 23.5±2.6 | 24.0±2.3 | 24.1±2.0 | 25.2±2.5 |
| | | 300 | 21.0±1.8 | 20.2±1.9 | 19.06±2.5 | 21.8±2.2 | 22.2±2.0 | 22.3±2.3 | 22.8±1.9 |
| | RV10333 | 100 | 22.3±1.4 | 21.9±1.6 | 20.74±1.7 | 23.3±1.5 | 23.5±1.3 | 24.4±1.2 | 25.3±0.9 |
| | | 300 | 22.6±2.7 | 21.5±2.9 | 20.31±3.6 | 23.4±2.8 | 23.7±3.0 | 24.4±3.3 | 25.8±2.6 |
| | RV10511 | 100 | 24.3±3.0 | 23.8±2.7 | 22.49±2.4 | 24.8±2.8 | 24.7±2.9 | 24.9±2.6 | 25.3±3.0 |
| | | 300 | 21.9±2.5 | 21.8±2.7 | 20.70±2.9 | 24.7±2.6 | 25.3±3.0 | 25.6±2.6 | 26.2±2.4 |
| | RV101201 | 100 | 24.9±3.2 | 24.2±2.8 | 22.86±3.0 | 24.6±2.5 | 26.6±3.1 | 26.4±3.0 | 27.9±2.7 |
| | | 300 | 21.2±2.3 | 21.2±2.7 | 20.08±2.4 | 22.9±1.8 | 24.3±1.9 | 24.5±1.8 | 25.6±2.0 |
| Group II | | 30 | 20.9±2.7 | 20.6±2.4 | 19.48±2.3 | 24.2±2.6 | 24.5±2.5 | 24.9±1.9 | 25.7±2.6 |
| Group III | | n/a | 22.0±2.7 | 21.9±2.8 | 20.70±2.4 | 20.9±2.7 | 21.1±2.9 | 20.8±3.1 | 19.8±2.7 |
| Group IV | | n/a | 20.4±3.1 | 20.8±2.7 | 21.30±2.9 | 24.1±2.5 | 24.4±2.9 | 25.1±3.0 | 25.5±2.8 |

Results are mean ± standard deviation (SD) for five mice. n/a not applicable

Percentage body weight gain was determined during the study period in order to give a clear insight of the change in weight (Figure 6.1). This was achieved by comparing with initial weight before induction of diabetes. The percentage increase (Figure 6.1) may be an indication of the extract with better properties in terms of maintaining the body functioning. After 14 days, the test mice had regained the body weight to near normal which was comparable with the normal control and metformin treated animals. On the other hand, the diabetic untreated mice reported a continuous percentage decrease in the weight an indication that diabetes affected their body functioning.

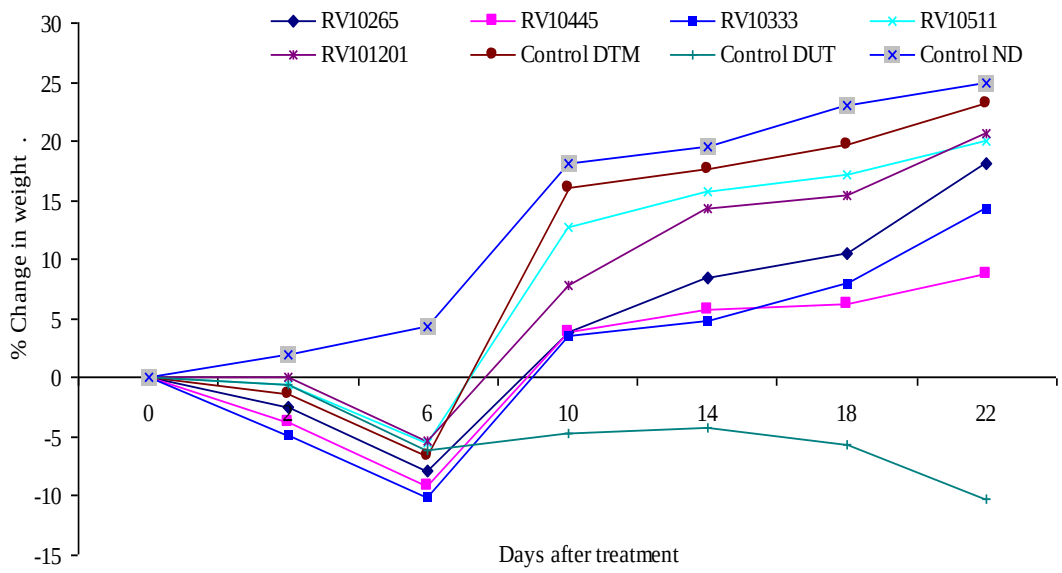



Figure 6.1: Mean percentage changes in body weights of diabetic mice treated with 300 mg/kg of African eggplant extract and 30 mg/kg metformin. Abbreviations represent DTM – Diabetic treated with Metformin, DUT – Diabetic Untreated and ND – Non-diabetic. RV10265, RV10445, RV10333, RV10511 and RV101201 represent Group I; control DTM represent Group II; Control DUT represent Group III; Control ND represent Group IV

### 6.4.2 Effects of African eggplant fruit extracts on blood glucose

After alloxan induction, the animals were characterized by the sustained elevation of blood glucose level which rose to approximately 13–18 mMol/L (2-3 times) within two days of induction; a confirmation that diabetes was induced (Trivedi *et al.*, 2004; Rahmatullah *et al.*, 2009; Sani *et al.*, 2009). These glucose levels were substantially greater than levels of normal non-diabetic mice (Group IV) which maintained almost constant levels of blood glucose which was within the normal range. Table 6.2 shows that the fasting blood glucose in non-diabetic mice (Group IV) levels ranged between 5.4 and 6.5 while alloxan-induced diabetic mice without any other treatment (Group III) it ranged between 5.9 and 19.5 during 22 days of experiment. Thus, glucose levels in the diabetic untreated mice greatly exceeded the normal upper limit and maintained the high levels. Aqueous fruit extracts of the tested African eggplant accessions significantly reduced ($p < 0.05$) the glucose level in the diabetic mice by the end of the experimental period, and greater glucose reduction was observed at 300mg/Kg than 100mg/Kg of the extracts. The results showed that there was a significant difference ($p < 0.05$) in glucose levels across the groups. The Tukey multiple comparison test showed that on day 18, there was

no statistically significant difference ($p > 0.05$) in the glucose levels of the normal mice and diabetic mice treated with metformin, RV10511, RV10333 and RV10265 extracts at 300mg/Kg. Generally, the diabetic untreated mice and diabetic control mice showed significantly higher ($p < 0.05$) fasting blood glucose when compared with normal control mice. Treatment of diabetic mice with doses of the fruit extract resulted in significantly lower ($p < 0.05$) levels of fasting blood glucose.

Table 6.2: Mean glucose levels (mMol/L) of alloxan-induced diabetic male BALB/c mice at different days

| | | **Conc (mg/Kg)** | **Days after treatment** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | **0** | **2** | **6** | **10** | **14** | **18** | **22** |
| Group I | RV10265 | 100 | 7.3±0.8 | 16.7±1.4 | 15.2±1.0 | 14.9±2.1 | 14.5±1.7 | 13.7±1.0 | 12.7±1.2 |
| | | 300 | 5.8±0.4 | 15.2±1.1 | 14.6±1.5 | 12.5±1.7 | 12.2±1.2 | 10.0±1.1 | 9.0±0.7 |
| | RV10445 | 100 | 6.1±0.7 | 17.9±1.0 | 16.8±1.1 | 15.8±0.9 | 15.3±1.3 | 14.5±0.8 | 13.7±1.3 |
| | | 300 | 5.5±0.2 | 13.7±1.9 | 13.1±0.9 | 12.9±1.4 | 12.2±1.0 | 12.5±1.2 | 11.4±0.9 |
| | RV10333 | 100 | 7.4±0.6 | 17.9±0.9 | 15.7±0.5 | 14.7±0.6 | 14.4±0.7 | 14.4±1.1 | 13.6±1.1 |
| | | 300 | 6.8±0.5 | 16.5±0.6 | 12.9±1.4 | 12.2±1.0 | 11.3±1.4 | 10.2±0.2 | 9.1±0.7 |
| | RV10511 | 100 | 5.3±0.0 | 13.3±1.3 | 12.4±1.1 | 12.5±1.3 | 12.2±1.8 | 10.5±1.4 | 10.4±0.9 |
| | | 300 | 5.9±0.2 | 14.7±1.7 | 12.3±0.8 | 11.7±0.5 | 11.0±0.9 | 10.1±1.0 | 8.6±0.8 |
| | RV101201 | 100 | 6.1±0.5 | 15.8±0.7 | 14.9±1.6 | 13.4±1.1 | 12.7±1.1 | 11.1±0.8 | 10.4±0.5 |
| | | 300 | 5.8±0.3 | 15.3±1.1 | 12.9±0.6 | 11.5±1.7 | 10.4±0.9 | 10.3±0.6 | 9.9±0.7 |
| Group II | | 30 | 6.6±0.4 | 14.3±0.6 | 12.4±.1.0 | 12.0±0.4 | 10.6±1.2 | 9.3±1.0 | 7.4±0.9 |
| Group III | | n/a | 5.9±0.5 | 15.3±1.0 | 15.2±0.9 | 15.8±1.0 | 17.8±1.9 | 18.2±2.1 | 19.5±2.3 |
| Group IV | | n/a | 5.4±0.9 | 6.5±0.2 | 6.1±0.5 | 4.8±0.3 | 5.9±0.3 | 6.4±0.6 | 5.6±0.5 |

Results are mean ± standard deviation (SD) for five mice. n/a- not applicable

Given that the glucose levels at the start of treatment varied considerably among the different groups of mice, the percentage reduction in glucose levels was calculated to obtain a better idea of the hypoglycemic efficacy of each of the treatments. The percentage change in blood glucose levels over the experimental period are presented in Figure 6.2. After the first 4 days of treatment, the concentrated extracts (300 mg/kg bwt) of accessions RV10333, RV10511, RV101201 and metformin recorded the greatest reduction in blood glucose levels 21.26, 15.97, 15.72 and 13.69 %, respectively, from the mean baseline glucose values. This was followed by RV10445 (4.82 %), RV10265 (2.55 %). At the end of the experiment, the mean glucose levels of metformin and extract treated animals were slightly above that of non-diabetic animals. However, metformin treated mice recorded the biggest fall in blood glucose levels (48.08 %) as

compared with the extract treated groups. It was followed by RV10333 (44.86 %), RV10511 (41.66 %), RV10265 (40.01 %), RV101201 (35.23 %), and RV10445 (17.23 %). From the results, it is evident that effects of RV10333, RV10511 and RV10265 fruit extracts were comparable with the conventional drug, metformin.

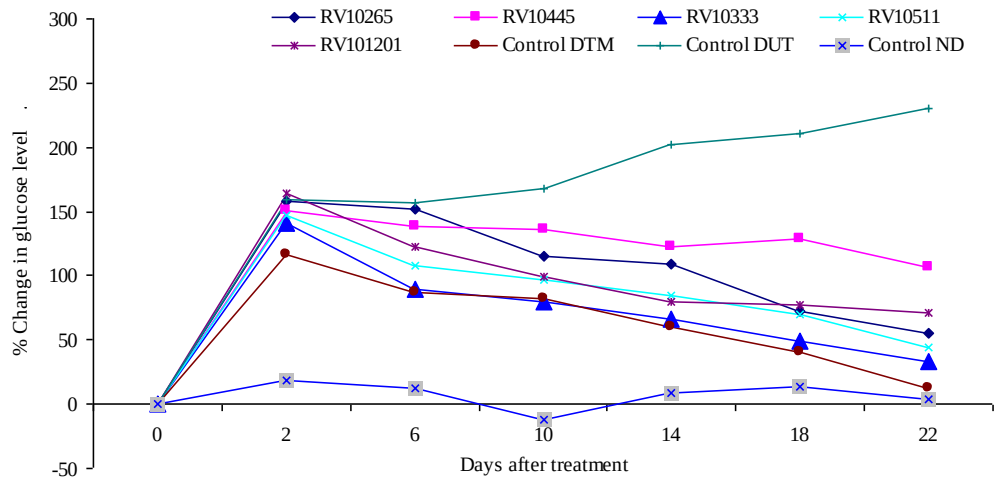



Figure 6.2: Mean percentage changes in blood glucose levels of diabetic mice treated with 300 mg/kg of African eggplant extract and 30 mg/kg metformin. Abbreviations represent DTM – Diabetic treated with Metformin, DUT – Diabetic Untreated and ND – Non-diabetic. RV10265, RV10445, RV10333, RV10511 and RV101201 represent Group I; control DTM represent Group II; Control DUT represent Group III; Control ND represent Group IV

### 6.4.3 Effects of African eggplant fruit extracts on biochemical parameters

The results Table 6.3 presents the change in biochemical parameters with the different treatment groups. From the results it was observed that Group III animals reported significantly high ($p < 0.05$) levels of bilirubin 20.63±3.40triglycerides (4.59±0.33), ALT (82.20±7.59) and urea (19.81±2.28) whereas hemoglobin was seen to be low in this group. On the other hand, there was no significant difference ($p > 0.05$) between the parameters of groups II and IV. For the extracts, there was no significant difference ($p > 0.05$) between the bilirubin and ALT levels of Group I (all the extracts) and the Group II and IV treatments.

Table 6.3: Mean levels of biochemical parameters of alloxan-induced diabetic male BALB/c mice treated with African eggplant extract and metformin after the treatment

| | Sample | Conc (mg/Kg) | **Biochemical parameters** | | | | |
|---|---|---|---|---|---|---|---|
| | | | **Bilirubin** | **Hemoglobin** | **Triglycerides** | **ALT** | **Urea** |
| Group I | RV10265 | 100 | 9.63±0.78$^{b}$ | 9.52±1.02$^{a}$ | 2.14±0.01$^{b}$ | 59.07±2.13$^{c}$ | 8.92±0.87$^{c}$ |
| | | 300 | 9.95±1.33$^{b}$ | 8.29±1.58$^{b}$ | 1.35±0.29$^{d}$ | 64.40±5.43$^{c}$ | 8.43±2.33$^{c}$ |
| | RV10445 | 100 | 8.55±1.04$^{b}$ | 8.28±0.54$^{b}$ | 3.33±0.54$^{b}$ | 70.38±4.26$^{b}$ | 11.24±1.12$^{b}$ |
| | | 300 | 10.31±0.14$^{b}$ | 8.16±4.44$^{b}$ | 2.55±0.26$^{c}$ | 70.08±3.68$^{b}$ | 10.43±1.94$^{b}$ |
| | RV10333 | 100 | 9.60±1.43$^{b}$ | 8.38±0.25$^{b}$ | 2.52±0.36$^{c}$ | 63.62±0.96$^{c}$ | 7.18±1.05$^{d}$ |
| | | 300 | 9.79±2.48$^{b}$ | 9.66±1.26$^{a}$ | 1.47±0.22$^{d}$ | 57.26±3.19$^{c}$ | 6.58±0.69$^{d}$ |
| | RV10511 | 100 | 8.63±0.56$^{b}$ | 8.47±0.54$^{b}$ | 2.37±0.13$^{c}$ | 59.78±2.54$^{c}$ | 7.26±0.43$^{d}$ |
| | | 300 | 10.38±2.58$^{b}$ | 9.61±1.45$^{a}$ | 1.93±0.37$^{cd}$ | 58.43±3.32$^{c}$ | 6.62±1.45$^{d}$ |
| | RV101201 | 100 | 9.16±1.12$^{b}$ | 10.70±0.96$^{a}$ | 2.42±0.08$^{c}$ | 58.27±2.33$^{c}$ | 9.26±1.17$^{c}$ |
| | | 300 | 9.63±0.78$^{b}$ | 9.61±4.05$^{a}$ | 1.71±0.38$^{d}$ | 62.22±9.16$^{c}$ | 9.24±0.71$^{c}$ |
| Group II | | 30 | 8.88±0.68$^{b}$ | 10.05±1.18$^{a}$ | 1.37±0.51$^{d}$ | 65.78±6.28$^{c}$ | 8.74±1.45$^{c}$ |
| Group III | | n/a | 20.63±3.40$^{a}$ | 8.30±1.95$^{b}$ | 4.59±0.33$^{a}$ | 82.20±7.59$^{a}$ | 19.81±2.28$^{a}$ |
| Group IV | | n/a | 8.65±0.14$^{b}$ | 11.85±0.95$^{a}$ | 1.63±0.17$^{d}$ | 57.87±7.84$^{c}$ | 7.80±1.48$^{d}$ |

Results are mean ± standard deviation (SD) for five mice. Means with different superscript letters within the column are significantly different ($P < 0.05$)

From the study, marked elevations of blood glucose were recorded immediately after alloxan administration. This was reduced when the diabetic mice was treated with the extract as well as the drug and the fruit extracts of RV10333, RV10511 and RV10265 accessions were comparable with the conventional drug (metformin). Regular administration of extracts for 21 days normalized lipid profile in diabetic animals. The data (Table 6.3) demonstrates that the alloxan induced diabetic mice had a slight increase in triglyceride value, as compared to the normal control mice. The mice treated with the extracts (at 300 mg/kg dose) except RV10445, together with metformin had significantly lower ($p < 0.05$) triglyceride values (< 2.0 µl/ml) which were not significantly different ($p > 0.05$) from those of the normal controls. On the other hand, the triglyceride values reduced in a significant manner with decrease in concentration. The mice treated with 100 mg/kg dose had slightly higher triglyceride values. This was comparable to those of the untreated group which had elevated values which was significantly higher ($p < 0.05$) than the normal control. On the other hand, the untreated diabetic mice (Group III) had elevated ALT levels as compared to the non-diabetic group. Oral administration of the RV101201, RV10333, RV10511 and RV10265 extracts or metformin also increased serum ALT as

compared to normal control mice, but these differences were not significant ($p > 0.05$). Interestingly, RV10445, which was the least effecting in lowering TG levels (Table 6.3), exhibited the highest ALT increase among the extracts (Table 6.3). The urea level for the untreated mice was significantly high ($p < 0.05$) whereas the levels for normal and treated mice were low. The difference in urea levels between the normal control mice and those treated with metformin or eggplant extracts (except RV10445) was insignificant ($p > 0.05$). In this study, the hemoglobin level of all the groups of mice including the untreated diabetic mice were between 8 – 12 mg/kg, and this was within the normal level.

## 6.5 DISCUSSION

From this study, it was observed that fruit extracts of RV101201, RV10333, RV10511 and RV10265 accessions at 300 mg/kg bwt had similar effects as metformin on body weight, plasma glucose levels and plasma triglyceride levels in mice. This may indicate the potential of these fruits in the management of diabetes. It was observed that, significant changes in parameters such as body weight, blood glucose level and biochemical parameters in the experimental mice may give a highlight on the antidiabetic activity of the test extracts. The loss of weight in diabetic mice might be contributed by decreased use of glucose by the tissues. This is because diabetes have been reported to cause failure to use of glucose for energy, therefore leading to increased utilization and decreased storage of protein responsible for reduction of body weight essentially by depletion of the body proteins (Guyton and Hall, 2000). The determination of the biochemical parameters in the blood of the experimental mice was useful in monitoring the adverse effects of the extracts on the body organs such as the liver, kidney and pancreatic. From the results, the extracts did not affect the biochemical parameters; bilirubin, ALT, urea and plasma triglycerides in mice. Bilirubin is the most abundant antioxidant in mammalian tissues and is responsible for most of the antioxidant activity in serum (Grover and Bafna, 2013). Its accumulation in blood could indicate defects in hepatic uptake, defects in hepatic excretion and hemolysis among other causes (Grover and Bafna, 2013) thus an indication of impaired liver function. From this study, no significant difference ($p > 0.05$) was observed between the bilirubin levels of the diabetic test mice treated with the extracts and metformin and the normal control mice. However the bilirubin level of the untreated diabetic mice (Group III) was slightly higher (2.5 fold) as compared with the non-diabetic control (Group IV). This indicates liver

damage in alloxan induced mice. The test extracts and metformin may have reversed this damage since the mice treated with these did not have increased bilirubin. The findings therefore suggest that the African eggplant extracts are hepatoprotective. The increased triglyceride levels in alloxan diabetic rats observed in the present study may be a result of increased breakdown of lipids and mobilization of free fatty acids from the peripheral depots (Poongothai *et al.*, 2011). This is because the breakdown of lipids gives free fatty acids and triglycerides which are then deposited in the plasma. These triglycerides also exhibited similar trends with glucose levels. Alanine aminotransferase (ALT) is a marker enzyme considered to be a true measure of liver function because it is synthesized only by the liver. Its level is an indicator of hepatocellular or liver damage where elevated levels indicate leakage from damaged heptocellular cells. Increased ALT is the most common abnormality in type 2 diabetes (Harris, 2005). In addition, diabetes mellitus affects the kidneys thus may lead to elevation of urea which is a marker of renal function (Poongothai *et al.*, 2011). It is believed that immediately after induction of diabetes, urea level significantly increased ($p < 0.05$) in all the diabetic mice and the administration of the extracts at dosage of 300 mg/kg and 100 mg/kg bwt, as well as metformin reversed and brought down the changes in urea to near normal levels, comparable to the normal control group.

Unlike the other extracts, the RV10445 extracts exhibited less antidiabetic efficacy, indicating that the antidiabetic activity of African eggplant is accession-dependent. The results of this study are in agreement with other studies that observed that antidiabetic potential of plant extracts are dependent on the plant (Abdirahman *et al.*, 2015; Chege *et al.*, 2015) and concentration (Grover and Bafna, 2013). The observed antidiabetic activity of African eggplant accessions may be attributed to their valuable bioactive metabolites with antioxidant properties including carotenoids and ascorbic acid and polyphenols (Borguini and Torres, 2009). These metabolites have been associated with hypoglycemic activity (Tan *et al.*, 2010). The presence of flavonoids has previously been reported to demonstrate hypoglycemic activity in streptozotocin induced diabetic male wistar rats (Piero *et al.*, 2015). Additionally, epidemiological studies and clinical studies have strongly supported the observation that adequate carotenoid supplementation significantly reduce the risk of several disorders such as cancers and diabetes which are mediated by ROS. Since carotenoids such as lycopene and β-carotene have been shown to act as powerful antioxidants in humans, a diet containing moderate amounts of these compounds has been

associated with the prevention of cardiovascular disease and diabetes (Rivera and Canela-Garayoa, 2012). Interestingly, the mice treated with the extracts except for RV10445 did not show any sign of clinical toxicity, stress and aversive behaviors during the treatment period. Further, no deaths as well as no change in body weight were observed during the study period, thus indicating that the aqueous extract of African eggplant fruits may be safe for consumption.

## 6.6 CONCLUSION

Although little was known concerning their therapeutic activity of African eggplant fruits against diabetes, this study has given an insight on their antidiabetic potential. From this study, it can be concluded that the extracts of RV10333, RV10511, RV101201 and RV10265 accessions showed antidiabetic activity thus are potentially important in management of diabetes. Furthermore, the extracts at high dose of 300 mg/kg bwt demonstrated antidiabetic activity. This was well demonstrated in the body weight changes, blood glucose levels and biochemical parameters. Although the extracts of RV10445 accession did not show antidiabetic activity, the biochemical parameters were not affected therefore may still be effective at higher concentration. The activity of the extracts may be attributed to their bioactive antioxidative compounds such as ascorbic acid, β-carotene, lycopene and flavonoids as reported in the preceding chapters in the study. Since the treatment with the extracts did not cause any deaths nor induce any signs of toxicity, it was a clear demonstration that the African eggplant fruits could exert antidiabetic activities with minimum toxicity and therefore may be safe for consumption.

# CHAPTER SEVEN

# GENERAL CONCLUSION AND RECOMMENDATIONS

## 7.1 CONCLUSION

The study showed the functional metabolites in the diverse African eggplant and African tomato accessions. It is evident that mature leaves and ripe fruits provide considerably high amounts of ascorbic acid and carotenoids particular beta carotene and lycopene thus important to human health. The increased ascorbic acid content in these crops might also be used as a parameter for predicting the stress tolerant crops. Therefore RV100343, RV100199 and RV100265 African eggplant accessions and V1030380, V1006892 and V1035028 African tomato accessions may be perceived to be more tolerant as compared to the others.

The metabolite profiles of African eggplant and tomato accessions demonstrate that metabolic adjustments in response to stress not only depend on the growth and developmental stage or stress of the plant but also on the accession. Drought stress accumulates metabolites particularly proline, sucrose and trehalose and accessions with higher levels of these osmolytes are tolerance to abiotic stress. Therefore indicate that African eggplants and tomatoes develop adaptive strategies including alterations of metabolism to withstand abiotic stresses and to ensure their survival under the drought stress condition. It is perceived that, the presence of osmolytes may have prevented the stressed accessions from wilting and prolong their survival under stress. The presence of secondary metabolites, including flavonoids, particularly quercetin and kaempferol, naringenin and their derivatives together with chlorogenic acid, means that the African tomato fruits are important for nutritional and medicinal value. Therefore the study attests the importance of African eggplants and tomatoes in providing the much-needed dietary nutraceutical potential since they have substantial amounts of the important metabolites.

The nutraceutical potential of African eggplants is justified by the antidiabetic properties observed in the study. It is therefore evident that African eggplant fruits are important in the management of diabetes and they are safe for consumption without any toxic effects. In conclusion, this study illustrates the common effects associated with drought stress on African

eggplant and tomato functional diversity and quality characteristics. Therefore this knowledge if well exploited could unlock the potential for stress management for improved food security and sustainable livelihoods in Africa.

## 7.2 RECOMMENDATIONS

The pertinent recommendations that have been identified and suggested from this study include the need to:

1) Exploit both African eggplants and tomato for production of nutrient rich functional foods;
2) Increase consumption of African eggplant fruits in management of diabetes.
3) Exploit controlled level of drought stress, imposed through reduced irrigation to enhance quality attributes of the tomatoes and eggplants.

## 7.3 SUGGESTION FOR FURTHER RESEARCH

1) Combine metabolomics, transcriptomics and genomics to get insight on the genes responsible for metabolic process of osmolytes under drought stress;
2) Other factors (biotic and abiotic factors) likely to affect metabolite composition should be explored to define stress tolerance;
3) Further antidiabetic studies should be done using primate animals and different concentrations.

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# APPENDICES

## Appendix 1: Chemicals and reagents

The reagents methanol, isopropanol, glacial acetic acid, tetrahydrofuran, petroleum ether, ethyl acetate, and methyltert-butyl ether were purchased from Fischer Scientific (Fair Lawn, NJ, USA). N-methyl-N-[trimethylsilyl] trifluoroacetamide (MSTFA) and ribitol was purchased from Sigma-Aldrich (St. Louis, MO, USA). Alloxan, metaphosphoric acid, ascorbic acid were purchased from Kobian Kenya Ltd and biochemical assay kits from Roche Diagnostics (Mannheim, Germany) whereas Glucophage (metformin) from the pharmacy. All reagents were analytical or HPLC grade. All mobile phases and samples were filtered before use.

## Appendix 2: Table showing African eggplant and tomato accession abbreviations used in Chapter Five

| **(a) African eggplant** | | | | | | |
|---|---|---|---|---|---|---|
| | **Stress** | | | **Control** | | |
| | **Mature green (MG)** | **Breaker (BK)** | **Mature red (MR)** | **Mature green (MG)** | **Breaker (BK)** | **Mature red (MR)** |
| RV100343 | 1_1_1 | 2_1_1 | 3_1_1 | 4_1_1 | 5_1_1 | 6_1_1 |
| RV100201 | 1_1_2 | 2_1_2 | 3_1_2 | 4_1_2 | 5_1_2 | 6_1_2 |
| RV100332 | 1_1_3 | 2_1_3 | 3_1_3 | 4_1_3 | 5_1_3 | 6_1_3 |
| RV100445 | 1_1_4 | 2_1_4 | 3_1_4 | 4_1_4 | 5_1_4 | 6_1_4 |
| RV100259 | 1_1_5 | 2_1_5 | 3_1_5 | 4_1_5 | 5_1_5 | 6_1_5 |
| RV100265 | 1_1_6 | 2_1_6 | 3_1_6 | 4_1_6 | 5_1_6 | 6_1_6 |
| RV100432 | 1_1_7 | 2_1_7 | 3_1_7 | 4_1_7 | 5_1_7 | 6_1_7 |
| RV100327 | 1_1_8 | 2_1_8 | 3_1_8 | 4_1_8 | 5_1_8 | 6_1_8 |
| RV100330 | 1_1_9 | 2_1_9 | 3_1_9 | 4_1_9 | 5_1_9 | 6_1_9 |
| GBK50591 | 1_1_10 | 2_1_10 | 3_1_10 | 4_1_10 | 5_1_10 | 6_1_10 |
| **(b) African tomato** | | | | | | |
| V1005987 | 1_1_1 | 2_1_1 | 3_1_1 | 4_1_1 | 5_1_1 | 6_1_1 |
| V1006833 | 1_1_2 | 2_1_2 | 3_1_2 | 4_1_2 | 5_1_2 | 6_1_2 |
| V1005872 | 1_1_3 | 2_1_3 | 3_1_3 | 4_1_3 | 5_1_3 | 6_1_3 |
| VI005878 | 1_1_4 | 2_1_4 | 3_1_4 | 4_1_4 | 5_1_4 | 6_1_4 |
| V1002114 | 1_1_5 | 2_1_5 | 3_1_5 | 4_1_5 | 5_1_5 | 6_1_5 |
| V1050580 | 1_1_6 | 2_1_6 | 3_1_6 | 4_1_6 | 5_1_6 | 6_1_6 |
| V1002112 | 1_1_7 | 2_1_7 | 3_1_7 | 4_1_7 | 5_1_7 | 6_1_7 |
| V1050589 | 1_1_8 | 2_1_8 | 3_1_8 | 4_1_8 | 5_1_8 | 6_1_8 |
| V1006838 | 1_1_9 | 2_1_9 | 3_1_9 | 4_1_9 | 5_1_9 | 6_1_9 |
| V1006826 | 1_1_10 | 2_1_10 | 3_1_10 | 4_1_10 | 5_1_10 | 6_1_10 |
| V1006828 | 1_1_11 | 2_1_11 | 3_1_11 | 4_1_11 | 5_1_11 | 6_1_11 |
| V1005874 | 1_1_12 | 2_1_12 | 3_1_12 | 4_1_12 | 5_1_12 | 6_1_12 |
| V1030380 | 1_1_13 | 2_1_13 | 3_1_13 | 4_1_13 | 5_1_13 | 6_1_13 |
| V1006892 | 1_1_14 | 2_1_14 | 3_1_14 | 4_1_14 | 5_1_14 | 6_1_14 |
| V1035028 | 1_1_15 | 2_1_15 | 3_1_15 | 4_1_15 | 5_1_15 | 6_1_15 |