Research Article

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Metabolomic analyses to evaluate the effect of drought stress on selected African Eggplant accessions

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Abstract

BACKGROUND: Drought stress is one of the main abiotic stresses that affect crops. It leads to biochemical changes that can have adverse effects on plant growth, development and productivity. African eggplants are important vegetable and fruit crops reported to adapt and thrive well under drought stress. The diversified metabolites arising due to stress have not been well defined. A gas chromatographic–mass spectrometric metabolomic approach was applied to characterize the effect of drought stress on metabolites at different stages of growth. Nineteen accessions were selected for analysis and drought was imposed by withholding water until soil moisture reached 60% field capacity. Fresh leaf tissues were sampled before stress, 2 and 4 weeks after stress and metabolite profiling done.

RESULTS: Significant changes in metabolite content 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 be positively correlated with stress. Principal component analysis showed a clear discrimination between the different accessions, growth stages and stress/control conditions.

CONCLUSION: The results illustrate that drought stress has a significant impact on the concentrations of some metabolites, such as amino acids, sugars and organic acids, which may contribute to drought stress effects and tolerance.

Supporting information may be found in the online version of this article.

Keywords: stress tolerance; abiotic stress; drought stress; African eggplants; metabolite profiling

INTRODUCTION

A rich diversity of wild or indigenous vegetable species exists throughout Africa.[1] 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,[1] and have played an important historical role in traditional diets due to their value in human nutrition.[2]

African eggplants (*Solanum aethiopicum* L.) constitute important leaf and fruit vegetables in Africa.[1] They are the most economically important and valuable crops in the Solanaceae family whose leaves and fruits are consumed. Their leaves and fruits are appreciated for their bitter taste. This could be attributed to the presence of alkaloids (mainly glycoalkaloids and phenolic compounds) determining to a great extent their edibility.[3] There is increasing evidence that intake of their leaves and fruits reduces the incidence of chronic diseases, including diabetes and atherosclerosis.[4] These crops also provide an economic pillar upon which women's livelihood is supported in Africa.[5] African eggplants have also been reported to be rich in carotenoids,[6] and thus possess additional health/nutraceutical significance.

Despite African eggplant cultivars thriving in drought-prone areas, severe water stress reduces their productivity. Water stress affects physiological, biochemical and molecular processes, such as photosynthesis, respiration, translocation, ion uptake, metabolism and growth promoters.[7] Depending on the intensity of the water, plant growth and development can be affected, leading to functional damage and senescence of plant organs, reduction in growth and reduction in fresh and dry biomass production.[7] Drought stress also activates the production of reactive oxygen species (ROS),[8] which has toxic consequences.[9] In addition, drought stress affects plants by disrupting metabolic homeostasis, causing significant changes in the composition of plant chemistry[7] resulting from adjustment of metabolic pathways

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Table 1. List of selected African eggplants from the accessions provided by AVRDEC-ESA

S/No.	RVI code	Genus	Species	Name	Origin
1	RVI00343	*Solanum*	*aethiopicum*	CN012	Cameroon
2	RVI00199	*Solanum*	spp.	EX-DAR	Tanzania
3	RVI00201	*Solanum*	*aethiopicum*	GKK-AE-158	Malawi
4	RVI00332	*Solanum*	*aethiopicum*	RNL187-194	Burkina Faso
5	RVI00271	*Solanum*	*aethiopicum*	Line 87	Mali
6	RVI00445	*Solanum*	sp.	S0004	unknown
7	RVI00333	*Solanum*	*aethiopicum*	SANGAWILI	Mali
8	RVI00259	*Solanum*	*aethiopicum*	Line 55	Senegal
9	RVI00265	*Solanum*	*aethiopicum*	Line 21	Mali
10	RVI00273	*Solanum*	*aethiopicum*	Line 89	Mali
11	RVI00511	*Solanum*	*aethiopicum*	SENGEREMA 1	Tanzania
12	RVI00432	*Solanum*	spp.	N4	Unknown
13	RVI00246	*Solanum*	*aethiopicum*	Line 112	Unknown
14	RVI00328	*Solanum*	*aethiopicum*	LOCAL MALI	Mali
15	RVI00327	*Solanum*	*aethiopicum*	AUBERGINE BLANCHE	Mali
16	RVI00342	*Solanum*	*aethiopicum*	OFARIWA'A	Cameroon/Ghana
17	RVI00330	*Solanum*	*aethiopicum*	Local Gaya	Mali
18	GBK050591	*Solanum*	*aethiopicum*		Kenya
19	RVI00438	*Solanum*	*aethiopicum*	MM1308	Unknown

RVI-Accession registration code used in AVRDEC.

for acclimation. In response to this, plants have been known to utilize various defense mechanisms against the abiotic environmental challenges and potential secondary opportunistic biotic agents.[10]

Metabolomic studies have contributed 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.[11] The study 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.[12] The responses of many plant species to drought stress have been extensively studied,[13] and 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.[14]

Besides, more metabolomic studies on solanaceous species of crops have 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[15] and root-knot nematode infestation,[16] revealed change in metabolite levels. In addition, most studies have reported effects of drought stress on concentration of different metabolites, for example in tobacco,[17] tomato,[18,19] potato[20] and eggplant.[21] On the other hand, studies within sections of the wild Solanaceae suggest that species harbor high chemical diversity.[19] In spite of this, limited attention had been paid to studies covering drought stress at development stages of African eggplants.

This study therefore involves the application of metabolomics in determining the effect of drought stress on the metabolite composition of African eggplants at different developmental stages. From this study, the 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.

MATERIALS AND METHODS

Plant material

Seeds of 74 African eggplant accessions were obtained from gene banks at local and regional centers and institutes, which include Kenya Agricultural Research Institute (KARI), Gene Bank of Kenya and the World Vegetable Centre (AVRDC), Arusha, Tanzania. Of the 74 accessions, 19 were selected based on their morphological traits, including fruit size and weight, fruit shape, fruit length, flower color, leaf blade length and width (Table 1).

Chemicals

The reagents methanol, isopropanol and glacial acetic acid were purchased from Fischer Scientific (Fair Lawn, NJ, USA), with the exception of *N*-methyl-*N*-[trimethylsilyl] trifluoroacetamide (MSTFA) and ribitol, which were purchased from Sigma-Aldrich (St Louis, MO, USA). All mobile phases and samples were filtered before use.

Experimental site and treatments

Experiments were carried out in the greenhouse at the Boyce Thomson Institute for Plant Research, Cornell University, USA, 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 African eggplant accessions were germinated in trays and the seedlings transplanted after 4 weeks of germination and grown alongside each other. Normal irrigation was maintained before and 5 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 growth media using randomized complete block design with three replications. The experiment had two treatments: drought stress and control experiments. Drought stress treatments were initiated after 5 days of transplanting. This was achieved by stopping irrigation for a few days and soil moisture monitored every day using Delmhorst model KS-D1 digital soil

moisture tester (Delmhorst Instrument Co., Towaco, NJ, 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 L) to compensate for this. For the control treatment, continued watering with sufficient amount of water (normal irrigation) was maintained throughout.

Sample collection

Fresh leaves were sampled early in the morning at different ages: before stress, 2 weeks and 4 weeks after stress for metabolomic analysis. The materials were harvested from fully expanded leaves of each plant at each stage. The harvested leaf tissues were immediately plunged (snap-frozen) in liquid nitrogen to quench further metabolism. Afterwards, they were ground in liquid nitrogen and stored in 15 mL Falcon tubes at −80 °C. The frozen leaf tissues were later used for metabolomic analysis using gas–chromatography mass spectrophotometry (GC–MS).

Metabolite extraction and derivatization

Metabolite analysis by GC–MS was carried out by a method modified from Schauer *et al.*[19] and Ruprecht *et al.*[22] Frozen leaf tissues (100 mg) of each sample were weighed and transferred to 2 mL Eppendorf tubes and extraction solvent; methanol–isopropanol–glacial acetic acid (80:19:1, v/v) containing ribitol (1.25 μg mL^{-1} extraction solvent) as internal quantitative standard was added at a ratio of 10 μL mg^{-1} sample. Two beads were placed in each tube and vortexed or homogenized with a FastPrep machine (FastPrep-24, MP Biomedicals, Santa Ana, CA, USA) for 2 min and left to stand for 30 min. It was then centrifuged at 9408 × *g* for 15 min at room temperature. 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, and centrifuged for 2 min or until the solution was clear. 200 μL supernatant was transferred to a glass vial and evaporated under nitrogen to dryness. The residue was redissolved in 50 μL *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 h at 80–90 °C on a sand heat/bath. Derivatized samples were cooled to room temperature and then transferred by a glass syringe to a 200 μL glass insert and inserted into the same vial and tightly capped. A sample volume of 1 μL was injected into the gas chromatograph column for analysis.

GC–MS analysis

The GC–MS analyses were carried out on a Varian CP-3800 gas chromatograph coupled to a CombiPal autosampler (LEAP Technologies, Carrboro, NC, USA) and a Varian 1200 L triple-quadrupole mass spectrometer (Varian, Carey, NC, USA). The gas chromatograph was fitted with a VF-17 ms fused-silica, 30 m × 0.25 μm film thickness Factor Four gas chromatograph column (Varian). Helium was used as the carrier gas at a constant flow rate of 1 mL min^{-1}. The analysis was performed under the following temperature gradient program: 2 min isothermal heating at 70 °C, followed by 5 °C min^{-1} oven temperature ramp to 150 °C and held for 5 min; then to 300 °C at a rate of 10 °C min^{-1} 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) mass spectra were collected at 70 eV and mass spectra were recorded at 4 scans per second with an *m/z* 100–600 scanning range. Metabolite identification was carried out using 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, within each chromatogram the peak areas of the compounds were normalized by the sample fresh weight and by the peak area from the internal ribitol standard, resulting in relative response ratios for all compounds.

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 (14th edition). Principal 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 separate samples into clusters based on treatment and developmental stages was done using MetaboAnalyst software. Data processing and multivariate analysis was also performed using XCMS online (Scripps Research Institute, La Jolla, CA, USA) software (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.

RESULTS AND DISCUSSION

Drought-stressed crops were characterized by constant wilting (Fig. 1), which influenced the morphology of the different accessions. For example, leaf size and shape of the stressed accessions were reduced as compared to the control plants.

Detected metabolites

In the current study, a total of 29 compounds were successfully detected and identified, as shown in Table 2. In some cases, the metabolites were significantly lower, below detection limit or were not identified from the metabolite library. The detected compounds comprised three main groups: organic acids (14), sugars (8) and amino acids (7). Phosphoric acid was also identified in the samples. The results for each compound and individual African eggplant accession are presented as relative response ratios (see Supplementary Tables 1, 2 and 3) obtained by dividing the sample peak area by the peak area of the internal standard, ribitol.

Cluster analysis

The differences in metabolite levels in drought-stressed crops were correlated with the levels from the controls and presented by heat map (Fig. 2). 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 (Fig. 2). Block 1 indicates a group of metabolites that showed an increase in levels with stress; block 2 indicates a group of metabolites that showed a decrease in levels during stress; block

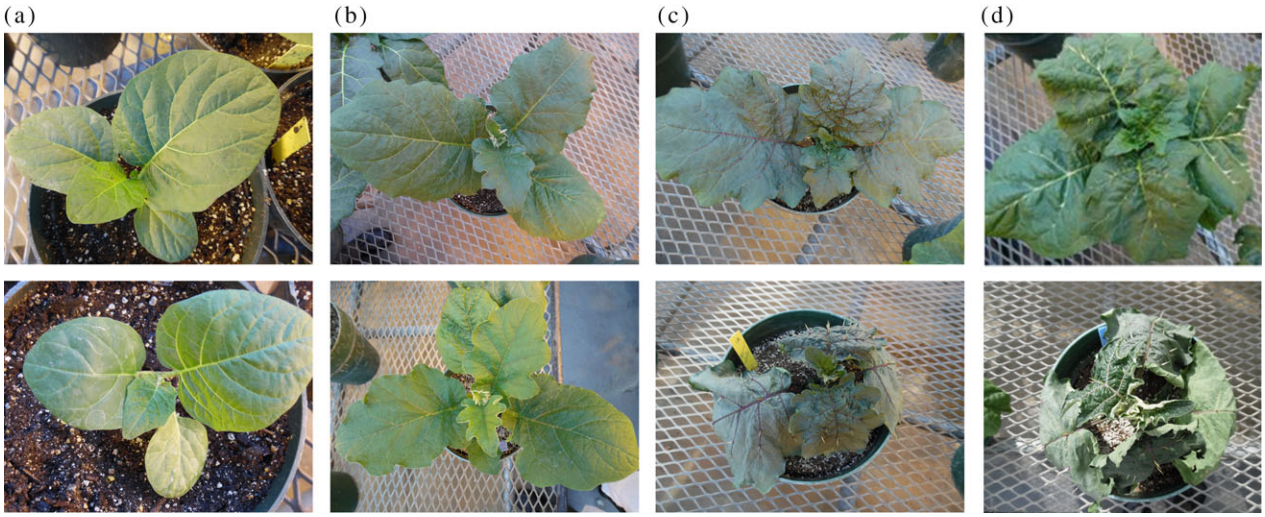

Figure 1. African eggplant (RV100332) accession. The top picture shows the control accession; the bottom picture shows the drought stress accession (a) immediately after transplanting, (b) before stress, (c) 2 weeks' stress and (d) 4 weeks after stress.

Table 2. Metabolites identified from African eggplant leaf extracts by GC–MS.

Organic acid	Sugar	Amino acid	Other
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			

3 indicates metabolites that showed no significant change; and block 4 indicates metabolites that were detected at low levels in most accessions. This analysis clearly positioned the different developmental stages and treatments in a chronological order with replicate samples positioning close together.

Application of PCA to the whole dataset using identified metabolites was aimed at finding the directions that best explain the variance in the dataset (Fig. 3). Principal component 1 (PC1) and principal component 2 (PC2) explained 72.4% and 15.5% of total variance (Fig. 3a). According to 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). This indicates that most of the variation in the metabolite composition of the crops can be attributed to differences in the developmental stage as well as the treatment. In addition, there was discrimination of metabolites between the accessions, drought-stressed and control crops, as well as at different developmental and growth stages. From 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 (Fig. 3b), proline, glutamate (Fig. 3c) and tricarboxylic acid (TCA) cycle metabolites such as citric acid, isocitric acid, fumaric acid and malic acid (Fig. 3d), among others. This is shown by loadings for variables far from the axis, and this indicates metabolites that have a high discriminating power. The data reveal 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 with the fourth week of drought stress.

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 a significant difference between the three different growth stages ($P < 0.05$) whereby an increase in the levels of sugars was observed during growth (Fig. 4). The sugar levels of individual accessions (see Supplementary Table 1) followed the same trends as the averages reported in Fig. 4. The major sugars observed in the African eggplant species were glucose, fructose, sucrose, trehalose and myo-inositol, as expected, whereas mannose, xylose and sorbose were of low abundance. 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 the exception of myo-inositol, significantly accumulated ($P < 0.05$). On the other hand, sorbose level was significantly high ($P < 0.05$) in week 2 as compared to week 4, with the control plants displaying significantly lower levels within the first 2 weeks but no detection at week 4.

Levels of individual sugars in the study varied considerably among the accessions, and within the treatment and developmental stage. An increase in most sugars was observed in both drought and control plants and subsequent accumulation at the advanced stage (week 4 of growth). This agrees with other studies reporting that older leaves are affected more by drought and accumulate higher amounts of metabolites, including sugars.[23]

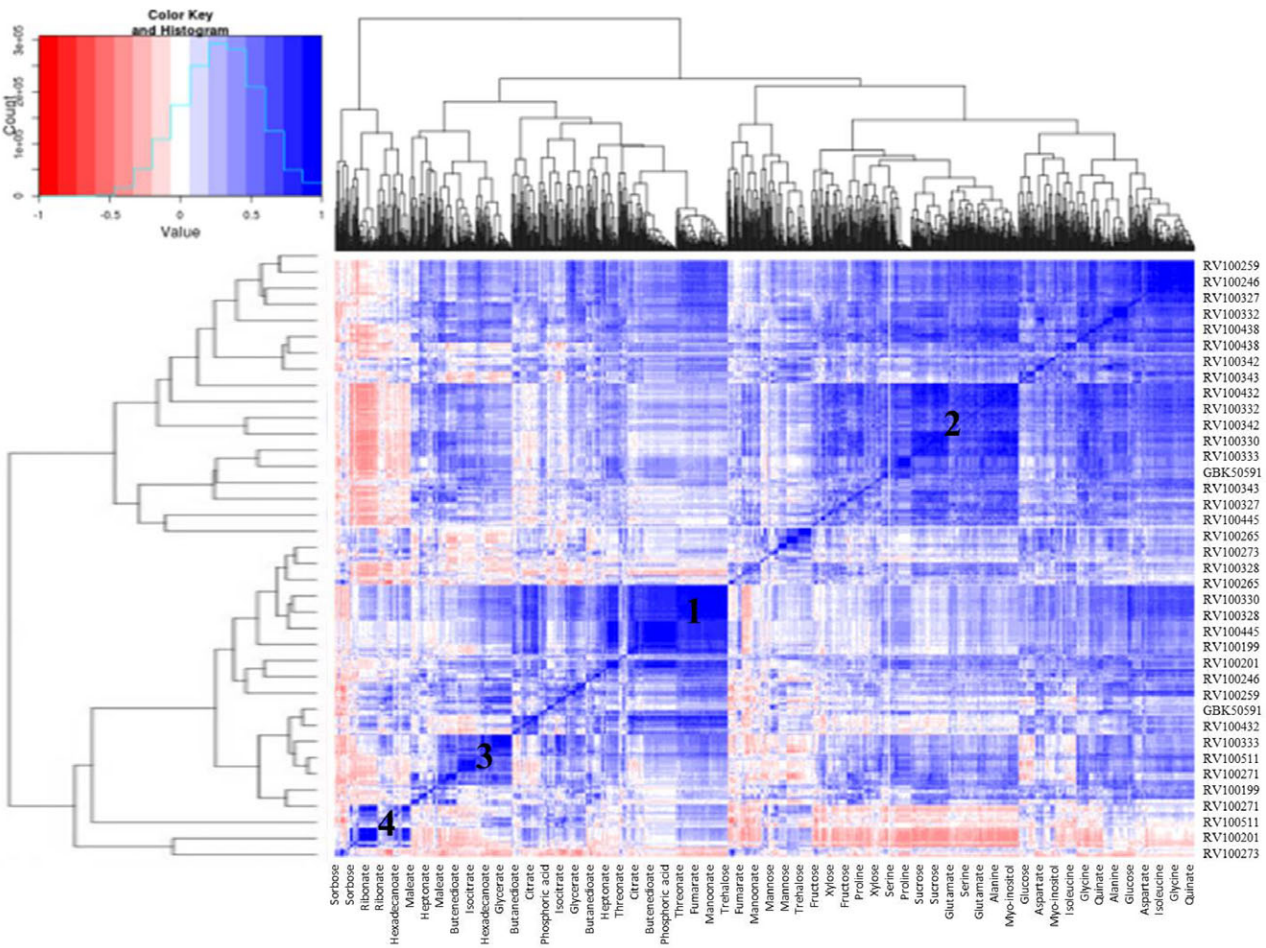

Figure 2. Combined cluster analysis of the metabolites of African eggplant leaf samples. Heat map showing correlation and cluster analysis of metabolite levels in leaves of different stressed and controlled African eggplant accessions at different growth and development stages. Samples were analyzed by GC–MS and the dataset comprised a total of 29 named metabolites. The marked blocks refer to strongly correlated metabolites that show similar behavior, and the color key indicates the extent of changes in metabolites.

Although significant increases were observed in the levels of sucrose, fructose, trehalose, mannose and xylose with stress and developmental stage, glucose levels were reduced. Glucose levels decreased in most of the drought-stressed accessions at week 2 and increased at week 4 as compared to control (see Supplementary Table 1). This contrasted with the findings of other studies,[24] which showed an increase in the levels of glucose in plants subjected to drought. Glucose is a product of photosynthesis in plants, a process dependent on water. Since drought stress is characterized by low water level, it confers a significant impact on the synthesis of glucose and other sugars. Many studies have been performed to assess the impact of drought stress on photosynthesis in developed leaves.[25] The effects of drought on photosynthesis have been reported to vary according to the intensity and duration of the stress, leaf age and plant species. Therefore, it is likely that the decreased level of glucose at week 2 is induced by reduction of photosynthesis. According to Chaves *et al.*[23] the rate of photosynthesis usually decreases during exposure to various stresses in higher plants.[23] Reduction of photosynthesis arises due to the affected photosynthetic pigments, damage to photosynthetic apparatus, decrease in leaf expansion, impaired photosynthetic machinery and premature leaf senescence. These pigments include chlorophylls, which are responsible for the synthesis of sugars by plants and adversely affected during stress.[26] Mibei *et al.*[6] reported reduced chlorophyll concentration in African eggplants subjected to stress, which might explain at least partly the decreased glucose under drought stress. There was a later increase of glucose levels in drought-stressed accessions at week 4. Although the mechanism behind this increase at a later stage is not well understood, Chaves *et al.*[23] reported that growth stage is more sensitive to water stress than photosynthesis, and in most cases mature leaves are more likely affected by drought and accumulate a large quantity of osmolytes as compared to young leaves. Whereas most of the accessions reported a significant decrease in glucose levels during stress at week 2 as compared to controls, the 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. Although the same correlations have been reported in different plant species,[14] it has been recently recognized that tolerance is also achieved by reducing growth and stomatal conductance, thereby slowing water loss and the onset of drought.[27]

The results of our study concur with the findings of other studies that have reported accumulation of a variety of sugars such as glucose, fructose, sucrose and trehalose in many crops

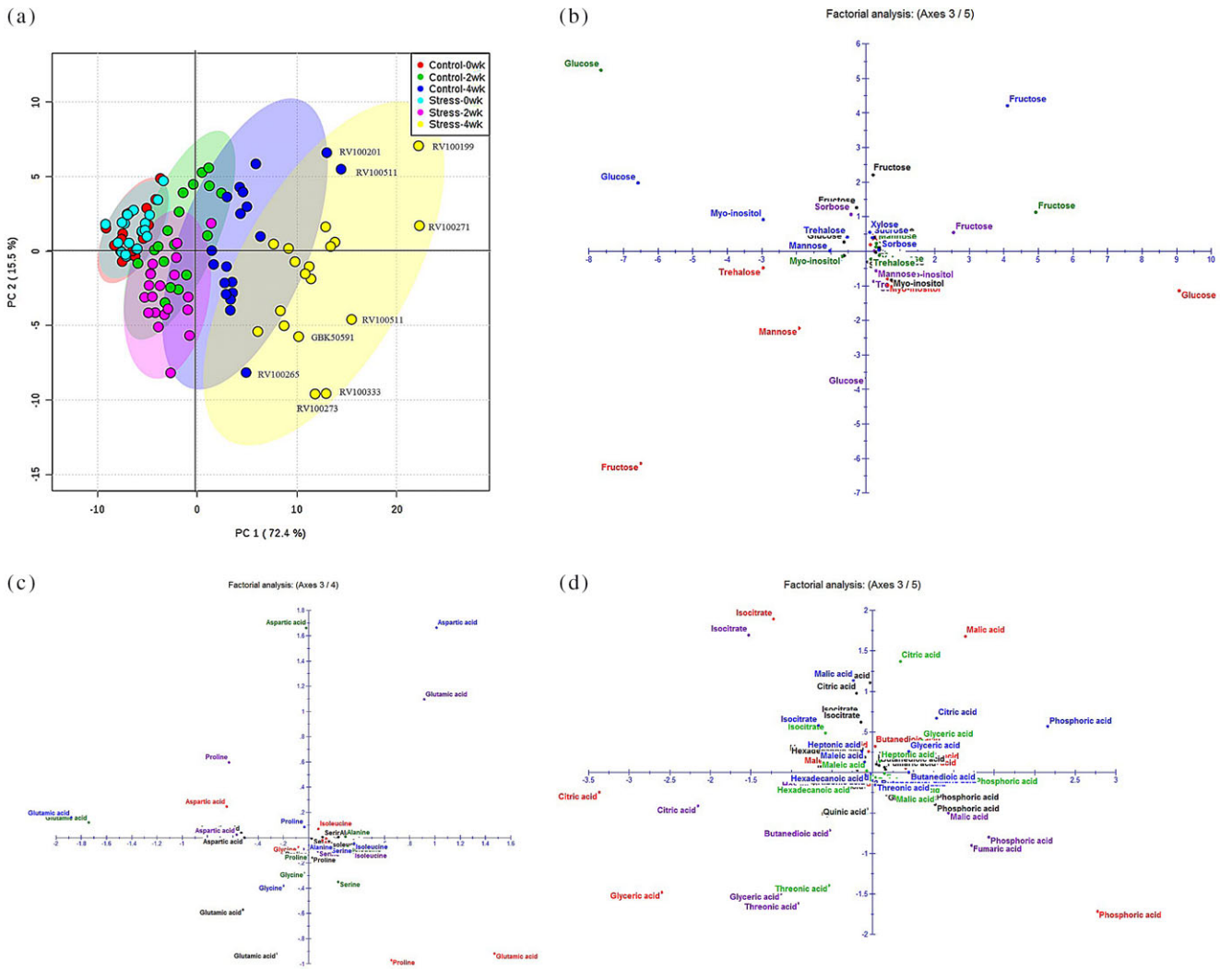

Figure 3. PCA of metabolic profiles in leaf samples of African eggplant accessions under control and drought stress conditions at different stages of development (three biological replicates). The data matrix consisted of 19 samples and 29 identified metabolites: (a) unsupervised PCA score scatter plot of metabolite profiles of samples of the African eggplant accessions; control and drought stress at different developmental stages; (b) sugars; (c) amino acids; (d) organic acids under control and drought stress conditions. The control and drought-stressed accessions showed clear separation in PC1. Colors represent: black, 0 weeks' stress and control; purple, 2 weeks' stress; green, 2 weeks' control; red, 4 weeks' stress; blue, 4 weeks' control.

in response to drought stress. This likely represents an adaptive response that helps plants cope with drought stress via osmotic and non-osmotic mechanisms. For example, increases in fructose and glucose contribute to osmotic adjustment.[28] Rizhsky *et al.*[24] reported that plants subject to a combination of drought and heat stress accumulated sucrose and other sugars, including maltose and glucose. Sicher *et al.*[29] reported increased carbohydrates, including sucrose, raffinose, glucose, fructose and maltose, and decreased levels of myo-inositol in water-stressed barley roots. In addition, severe water stress has been reported to be accompanied by accumulation of sugars such as sucrose.[30] Moreover, trehalose related to stress tolerance in bacteria and fungi has also been reported to accumulate in plants and may play a protective role during abiotic stress.[31] These soluble sugars are osmolytes, which function as osmoprotectants during water deficit.[28] They also play an 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.[28] On the other hand, changes in xylose, a key component of cell walls, suggest that an additional way the African eggplants may cope with drought stress is through cell wall modification, as has been observed in other species.[32]

Our 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.[33]

Effect of drought stress on amino acid levels in the leaves of African eggplant accessions

Figure 5 provides varying levels of amino acids in the stressed and control plants as well as at different growth and developmental stages from 0 to 4 weeks. Relatively high accumulation of aspartate, glutamate and proline was observed, with relative response ratios ranging from 0.3 to 5. On the other hand, significantly lower levels of amino acids serine, isoleucine and alanine were observed in the leaves of all accessions. Glycine was moderately high as compared to these amino acids.

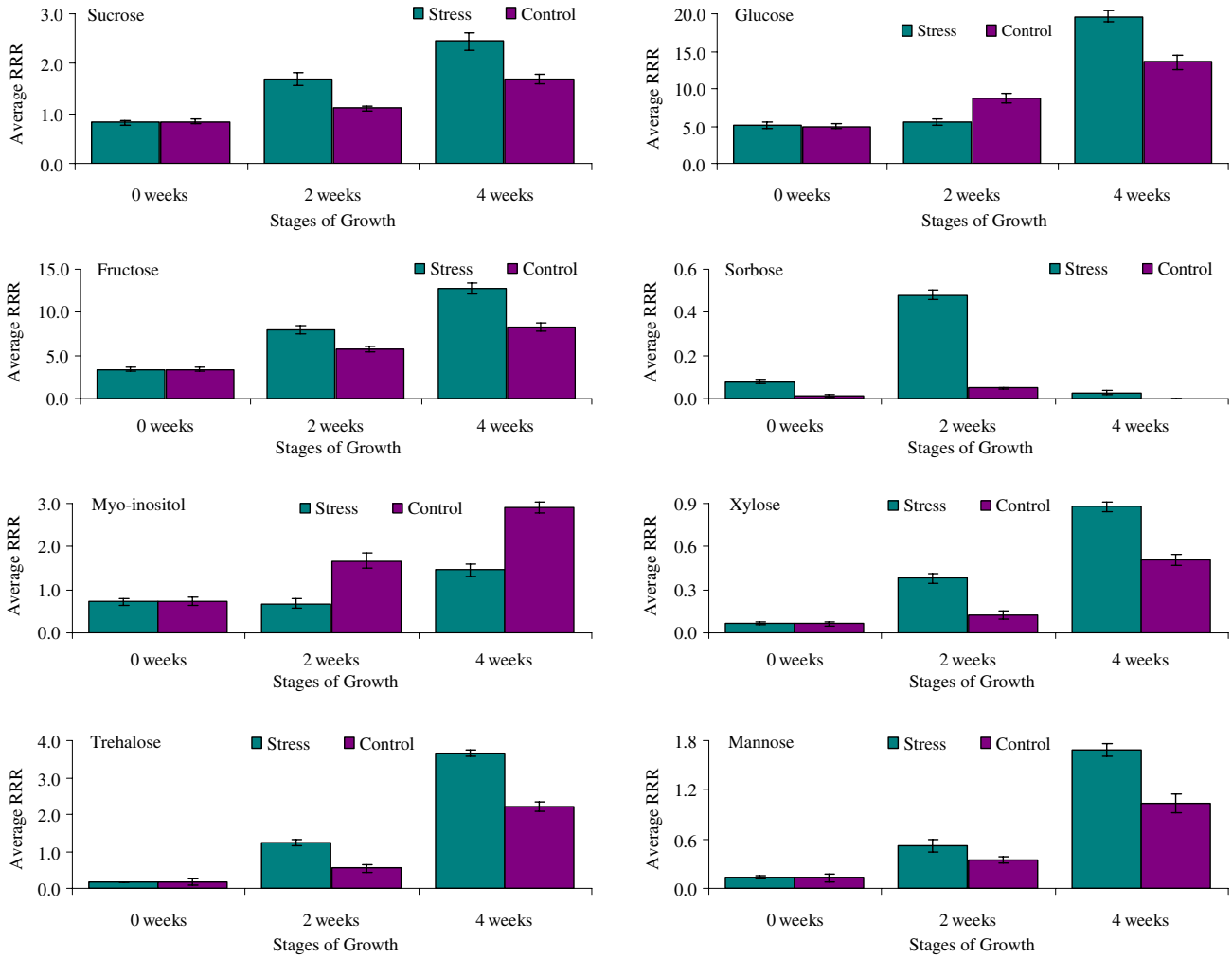

Figure 4. Average relative response ratios (RRR) of sugars in African eggplant accessions at different growth and development stages during stress. The RRR were obtained by dividing the peak area of the sample by the peak area of ribitol (internal standard). Data are mean values (of all 19 accessions) ± standard error. The compounds in the control and drought-stressed accessions are clearly visible in the charts.

The levels of proline and glutamate significantly increased ($P < 0.05$) during drought stress. An increase in proline and glutamate in the leaves at week 4 suggests an increased assimilation at the mature stage. On the other hand, isoleucine showed a significant increase at the second week of growth but decreased at week 4, while no significant difference was observed in isoleucine at week 4 between the drought-stressed and the control. Conversely, the contents of alanine, glycine and aspartate showed a significant decrease in the drought-stressed crops in all the accessions. Aspartate metabolism may have been inhibited under drought stress, resulting in its decrease. This suggests that the conversion of oxaloacetate to aspartate by aspartate aminotransferase (with the amino group donated by glutamate) may be limiting the flow of excess nitrogen into asparagine and may contribute to ammonia toxicity.[17] Similarly, pyruvate metabolism was also inhibited, causing a reduction of alanine and isoleucine. Despite the fact that the majority of identified amino acids increased during growth, and varied with stress, serine was fairly constant in all treatments.

Under drought stress, levels of the following amino acids were significantly high ($P < 0.05$) in the following accessions: proline (RV100273 and RV100432), glycine (RV100201, RV100445, RV100199), aspartate (RV100271, RV100333, RV100265, RV100342), glutamate (RV100343, RV100199, RV100332, RV100511) and alanine (RV100332, RV100265, RV100445). Some accessions had significantly lower levels of amino acids, while some amino acids remained fairly constant in all accessions even under drought (see Supplementary Table 2).

Drought stress leads to accumulation of ROS in plants that have the ability to react with proteins, lipids and DNA during abiotic stress to impair the normal function of cells.[9] Therefore, there is a high demand in water-deficit-treated plants to adjust osmotically and detoxify ROS. As a result, there is accumulation of common osmolytes and osmoprotectants, which helps in sustaining cell turgor by osmotic adjustment and stabilizing enzymes. This confers protection against oxidative damage to plants under extreme drought stresses. Accumulation of amino acids, therefore, has been suggested to aid stress tolerance in plants through osmotic adjustments and detoxification of ROS.[8] Proline has been specifically reported to be the most abundant osmolyte, and an ROS scavenger, synthesized in response to environmental stresses and which accumulates in response to drought.[31] Similarly, a decrease in intracellular pH leading to cytosolic acidosis has been associated with stress and linked to proline accumulation in plants.[34]

The results here agree with other findings that have reported accumulation of proline in many plant species in response to different environmental stresses, including drought.[35] The capacity

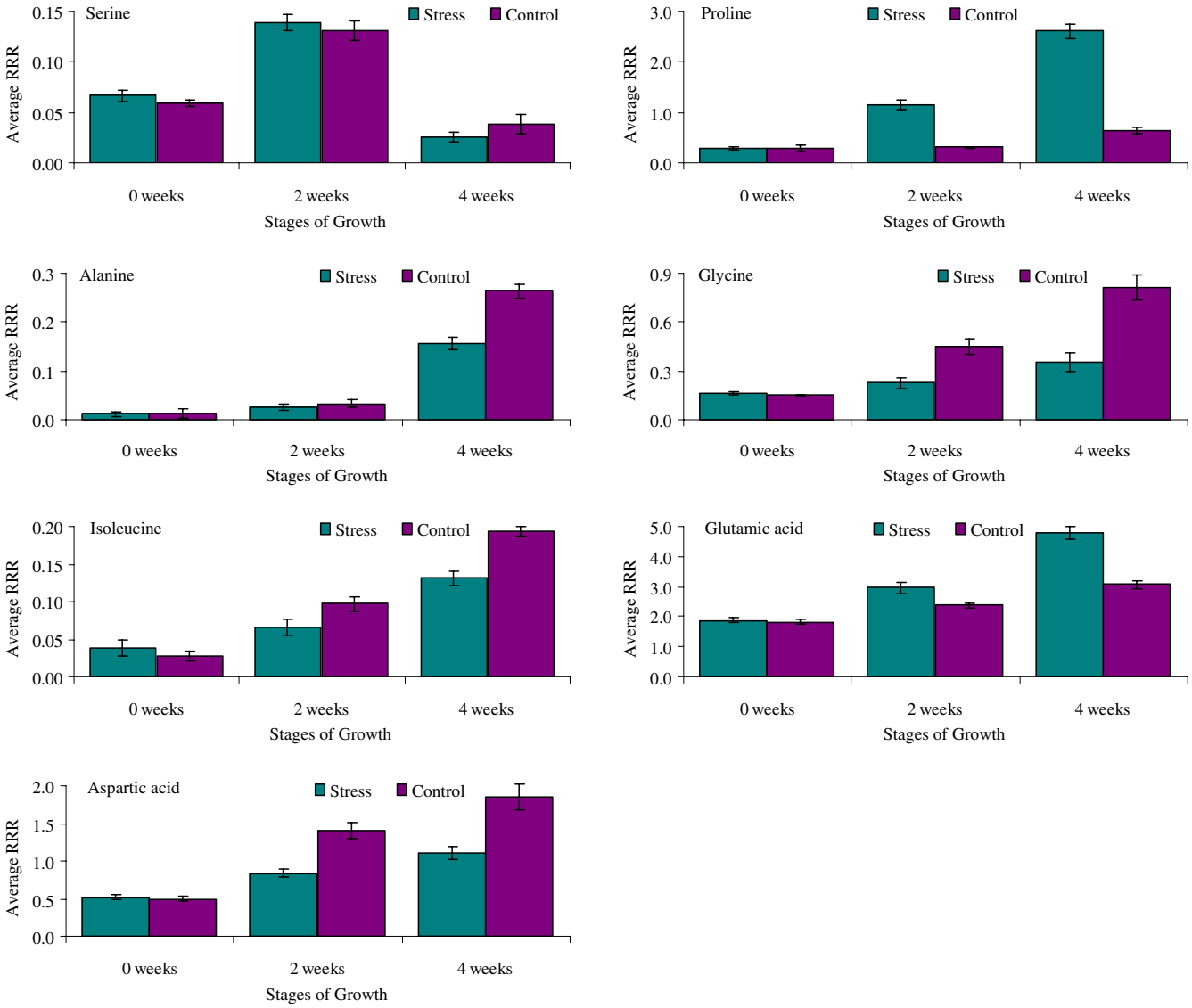

Figure 5. Average RRR of amino acids in African eggplant accessions at different growth and development stages during stress. This was obtained by dividing the peak area of the sample by the peak area of ribitol (internal standard). Data are mean values ± standard error. The compounds in the control and drought-stressed accessions are clearly visible in the charts. The significance of differences between control and drought-stressed accessions was determined by ANOVA.

of plants to accumulate proline has been correlated with increased tolerance toward water deficit, and therefore it is argued that proline plays a role in drought tolerance.[31] In addition, proline concentrations increase with reduced leaf water potentials, and therefore photosynthesis is quite reduced. Metabolic studies have revealed that proline level is determined by the balance between its biosynthesis and catabolism.[36] It is synthesized from glutamate (Fig. 6) in the chloroplasts to protect cell membrane and protein content in plant leaves, thereby protecting cells from damage caused by stress.[36] Proline is then degraded in mitochondria by proline dehydrogenase and pyrroline-5-carboxylate (P5C) dehydrogenase to glutamate. Therefore, its catabolism is enhanced during recovery from stress, leading to accumulation of glutamate. Glutamate is also a precursor of many stress-related compounds, and a higher pool of glutamate was hypothesized to lead to faster production of defense metabolites.[37] Accumulation of glutamate is also known to regulate and integrate the metabolism in stressed photosynthetic tissues.[23] This may explain increased glutamate in the African eggplants in response to drought stress, as reported in our study. On the other hand, there was a dramatic drop in aspartate and isoleucine during drought. Not all drought-associated metabolite changes were consistent in all species; for example, glycine and alanine were more abundant in the control plants compared with the drought-stressed plants. Increased levels of specific amino acids have a beneficial effect during stress acclimation and this might stem from amino acid production and/or from enhanced stress-induced protein breakdown.[17] Furthermore, the branched amino acid isoleucine also displayed high levels throughout plant growth and this has a role in respiration as an alternative substrate under stress.[38]

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

Comparison of the organic acids (Fig. 7) revealed no major qualitative differences between the stressed and the control tissues, whereas the quantitative responses of most compounds and

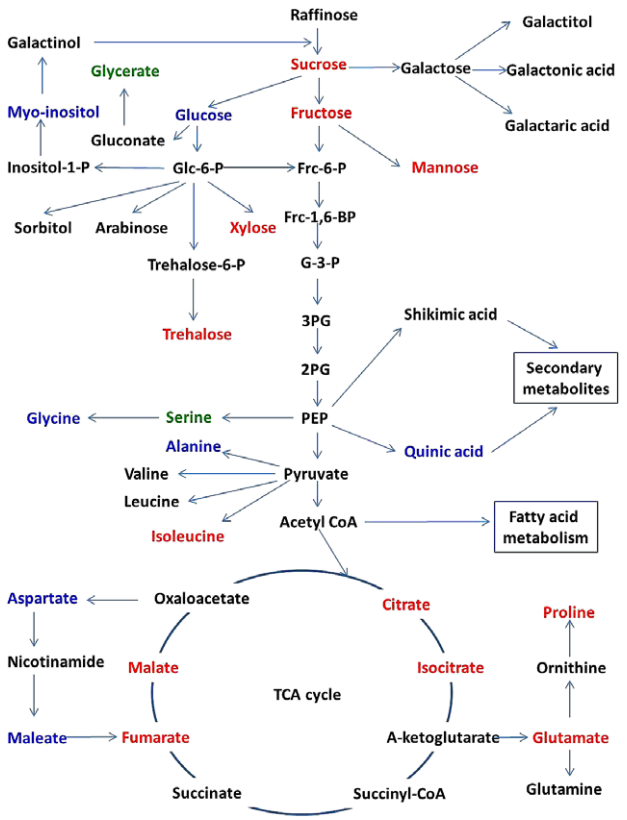

Figure 6. Metabolic pathway indicating the metabolites associated with the metabolism of various identified sugars, amino acids and organic acids in African eggplant accessions during stress and at different growth and development stages. Metabolites in black were not detected; green, detected but not significantly affected by drought stress; red, metabolites with greater relative response ratios (relative abundance) in drought conditions; blue, metabolites with lower relative abundance during stress.

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 phosphoric acid, an inorganic acid (see Supplementary Table 3). They mainly contributed to the differences in total organic acids. Citric, phosphoric and glyceric acids were the major ones, followed by malic, fumaric and isocitric acids and other less abundant acids (Fig. 7). 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 (Fig. 6). These TCA cycle metabolites, together with phosphoric acid, heptonic acid and butenedioic acid, were significantly increased in the stressed crops as compared to the controls, indicating that energy production in the 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. In addition, the stress response of the young leaves and mature leaves was distinct, with week 4 samples reporting significantly higher levels ($P < 0.05$) of most of the metabolites. Considerably lower levels were observed in threonic acid, butanedioic acid, quinic acid, butenedioic acid, heptonic acid, maleic acid and hexadecanoic acid. In contrast, no significant differences were observed in the levels of hexadecanoic, butanedioic, quinic and glyceric acids in the leaves of control and stressed accessions. Ribonic acid showed significantly lower levels in all the accessions as compared to other organic acids. The level was significantly lower or not detected in most accessions.

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 plant defense. The obvious role of quinic acid is as a precursor for phenolic compounds and other secondary metabolites.[39] 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.[40] Malate plays an important role as photosynthetic intermediate, an essential storage carbon molecule and as intermediate of the TCA cycle in all plant species.[40] In addition, recent evidence suggests that fumarate and malate play an important function as pH regulator and exhibit partial control over the efficacy of nutrient uptake and over stomatal function.[40] Previous studies found similar responses, with most organic acids and TCA cycle intermediates showing an increase in response to drought stress in different plant species.[41] Contrary to these are the findings of a study on wheat cultivars reporting that most organic acids decreased following drought stress.[28] Although the TCA pathway in plant is well known, its regulation is still poorly understood.[12]

In summary, drought stress has varied effects on the metabolic processes of leaves in the selected African eggplant accessions. Since some accessions were more tolerant compared to the others, the effects of drought on the metabolite levels was examined to determine whether any of the responses detected were related to known drought-stress-responsive metabolites. Comparison of the different African eggplant accessions under controlled and drought stress revealed many interesting changes in metabolite content and these were highly similar to those previously described.[14] It should be noted that there was varied content of the compounds with drought stress and, with progressing plant age, we found 4-week-old plants after stress (the oldest examined) being distinct. Perhaps metabolomics showed a greater difference between the stage of development and treatment than between distinct accessions, as reflected in the associated PCA (Fig. 3). 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, whereas 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. Water stress effects were observed for all the accessions through morphological responses, and major effects were noted with height and leaf size of the accessions, this being more characteristic at 4 weeks after stress. Initial morphological characteristics and metabolomic profiling of the accessions

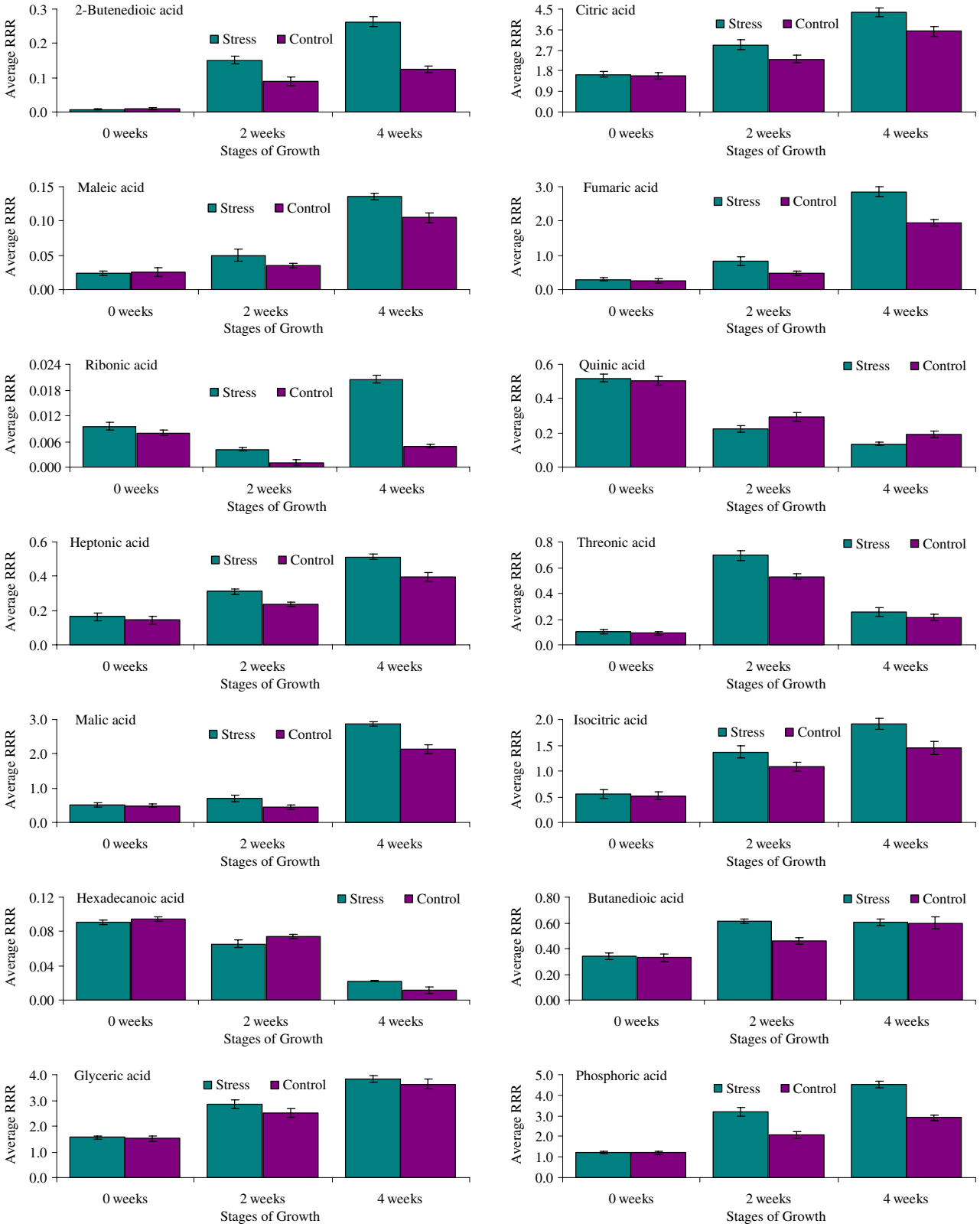

Figure 7. Average RRR of organic acids in African eggplant accessions at different growth and development stages during stress. This was obtained by dividing the peak area of the sample by the peak area of ribitol (internal standard). Data are mean values ± standard error. The compounds in the control and drought-stressed accessions are clearly visible in the charts. The significance of differences between control and drought stressed was determined by ANOVA.

uncovered putative metabolite components of the mechanisms responsible for an enhanced tolerance to drought stress. These components – specifically proline, sucrose and trehalose – have been reported to be osmolytes in other related studies and these were evident in our study. Therefore, the accessions with higher proline, trehalose and sucrose levels in drought stress displayed increased tolerance to abiotic stress. There was also an increased accumulation of glucose, fructose and glutamate under water stress, which was specific to the leaves of the more tolerant accessions. This indicates that these osmolytes prevent the stressed plants from wilting and prolong their survival under stress. Therefore, not only the wilting status of the crop but also the accumulation of metabolites may explain the differential drought tolerance observed here. Interestingly, the study showed that the more wilted the accession, the less the accumulation of the osmolytes and vice versa. Therefore, the decrease of osmolytes in accessions whose morphological characteristics indicated a higher stress levels seems to confirm the general view that accumulation of these metabolites contributes to stress tolerance. In conclusion, increase of osmolytes in our study is the major contributor to the observed drought resistance in the distinct accessions.

CONCLUSION

The analysis of metabolites contributes to the understanding of stress biology of plants through the identification of the compounds and the part they play in acclimation or tolerance response. Our study showed evidence of drought and oxidative stress responses in African eggplant accessions, as indicated by increased levels of compatible solutes such as sugars and amino acids as well as oxidative products of ROS remediation. The study displayed differences in the levels of organic acids, sugars and amino acids in response to drought stress and this was dependent on genotype. Seven important leaf metabolites (proline, glutamate, sucrose, fructose, glucose, trehalose and citric acid) were affected differentially among accessions when drought was imposed and were consistently increased or decreased in all African eggplant accessions upon drought stress. Some of the observed metabolite compositional changes are related to known phenomena associated with development, stress and photosynthetic activity. The findings of this study illustrate the common effects associated with drought stress on vegetable quality, and this involves stunted growth, leaf color, shape and size as well as change in metabolite composition. This work adds value to the study of stress tolerance and acclimation in crops and contributes to the understanding of the metabolic basis of stress biology in plants. Specifically, we provide evidence that proline, glutamate, sucrose, fructose, trehalose and citric acid are positively associated with stress tolerance in African eggplant and possibly other plants. Moreover, these data also provide information that may, with further experimentation, allow elucidation of the regulation of biochemical pathways underlying stress tolerance in African eggplants. In addition to stress tolerance, the observed metabolites may also contribute to the nutritional value of the leaves since amino acids and sugars are the precursors for nutritional biomolecules such as proteins and carbohydrates, respectively. These compounds may not only be of nutritional value but also of health and medicinal value. Therefore, African eggplants are a major source of biologically active nutritional substances and metabolites that are essential for plant growth, development, stress adaptation and defense as well as human health.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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