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Metabolic profiles of six African cultivars of cassava (*Manihot esculenta* Crantz) highlight bottlenecks of root yield

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SUMMARY

Cassava is an important staple crop in sub-Saharan Africa, due to its high productivity even on nutrient poor soils. The metabolic characteristics underlying this high productivity are poorly understood including the mode of photosynthesis, reasons for the high rate of photosynthesis, the extent of source/sink limitation, the impact of environment, and the extent of variation between cultivars. Six commercial African cassava cultivars were grown in a greenhouse in Erlangen, Germany, and in the field in Ibadan, Nigeria. Source leaves, sink leaves, stems and storage roots were harvested during storage root bulking and analyzed for sugars, organic acids, amino acids, phosphorylated intermediates, minerals, starch, protein, activities of enzymes in central metabolism and yield traits. High ratios of RuBisCO:phosphoenolpyruvate carboxylase activity support a C₃ mode of photosynthesis. The high rate of photosynthesis is likely to be attributed to high activities of enzymes in the Calvin–Benson cycle and pathways for sucrose and starch synthesis. Nevertheless, source limitation is indicated because root yield traits correlated with metabolic traits in leaves rather than in the stem or storage roots. This situation was especially so in greenhouse-grown plants, where irradiance will have been low. In the field, plants produced more storage roots. This was associated with higher AGPase activity and lower sucrose in the roots, indicating that feedforward loops enhanced sink capacity in the high light and low nitrogen environment in the field. Overall, these results indicated that carbon assimilation rate, the K battery, root starch synthesis, trehalose, and chlorogenic acid accumulation are potential target traits for genetic improvement.

Keywords: cassava, root yield, source/sink limitation, photosynthesis, carbon fixation, enzyme activity, nitrogen metabolism, starch synthesis, K battery, chlorogenic acid.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a perennial woody shrub of the Euphorbiaceae family and a staple food for over a billion people in Africa, Latin America and Asia (Chetty *et al.*, 2013; De Souza *et al.*, 2017). In Africa, cassava is the second most important calorific source with its tuberous roots often providing over a quarter of the daily

calorie consumption (De Souza *et al.*, 2017), and even more for the rural poor (<http://www.fao.org/publications/sofa/2016/en/>). In many countries of sub-Saharan Africa cassava leaves also provide an important source of protein, vitamins, and micronutrients for humans and livestock (Lukuyu *et al.*, 2014; Latif and Muller, 2015).

Worldwide cassava harvest expanded by 60% between 2000 and 2013 due to increased average yields per hectare (Howeler *et al.*, 2013). The latest FAO statistics revealed that cassava has overtaken soybean and potato in terms of calorific yield (<http://www.fao.org/publications/sofa/2016/en/>).

Despite extensive research on disease susceptibility and post-harvest deterioration, relatively little information is currently known about metabolism and source–sink interactions in this important crop. Indeed fundamental questions such as the mode of photosynthesis, how sucrose is unloaded in storage tubers and how the use of nutrients in growth is balanced with the requirement for anti-pathogenic cyanoglucosides while growing on impoverished soils remain unanswered or, for the first question, were only recently answered (Arrivault *et al.*, 2019). Earlier publications varyingly claimed that cassava is either a C₃ (Calatayud *et al.*, 2002), a C₄ or a C₃–C₄ intermediate plant (Cock *et al.*, 1987; El-Sharkawy and Cock, 1987), with the latter claims seemingly largely made due to the high levels of foliar malate and elevated activities of the C₄ enzyme phosphoenolpyruvate (PEP) carboxylase (El-Sharkawy, 2004). However, cassava does not display Kranz anatomy (El-Sharkawy, 2004) and archaeological isotope dating on conserved stomach contents gives consistent ages for cassava to those for plants that have been well established to employ the C₃ mode of photosynthesis. To directly address this question, ¹³CO₂ feeding experiments were performed on cassava and the metabolic fate of the ¹³C followed by mass spectrometry and shown to be very similar to the C₃ plant *Arabidopsis* (Szecowka *et al.*, 2013) and different to the C₄ plant maize (Arrivault *et al.*, 2017).

Crop yield can be limited by the ability to carry out photosynthesis and incorporate carbon dioxide into carbohydrates and other metabolites, or by the ability to utilize these resources for growth of storage organs. These are often termed ‘source’ and ‘sink’ limitation, respectively. The contribution of source and sink limitation to yield has been mainly researched in temperate crops. There is considerable evidence that source and sink can co-limit yield to a varying extent, and that their contribution varies with developmental stage and environmental conditions, including the availability of light, water, and nutrients (Sonnewald and Fernie, 2018), as well as the plant architecture including the leaf area ratio (White *et al.*, 2016). In cassava, our poor current understanding of basic fundamental aspects of metabolism and development makes it difficult to assess the extent to which yield is source and sink limited. It is also possible that the impact of developmental stage and environment may be more complex than in temperate crops. For example, under conditions of extreme drought cassava shed their leaves and later, when water becomes available again, remobilize reserves from the storage roots to make new leaves (El-Sharkawy, 2004).

Cassava is known to be reasonably productive even on unfertilized poor soils (El-Sharkawy, 2012). This renders cassava a suitable staple crop for resource-limited small-holders to cultivate for successive seasons on low fertility soil. Productivity on low nitrogen soil is partly attributed to high photosynthetic nitrogen-use efficiency (El-Sharkawy and De Tafur, 2010). A positive correlation is observed between photosynthetic nitrogen-use efficiency and root yield in different cassava genotypes (El-Sharkawy and De Tafur, 2010). However, the metabolic characteristics that enable cassava to grow under such conditions remain unclear. Also, although cassava is tolerant to low fertility soil, fertilization significantly improves root yield (Fermont *et al.*, 2009) indicating that nitrogen availability can be a limiting factor for cassava root production. Indeed, cassava can consume a vast amount of soil N with some 180 kg N ha⁻¹ being required to produce 30 t ha⁻¹ tuberous roots (Byju and Anand, 2009). High nitrogen fertilization is unaffordable to many small-holders and therefore is rarely applied in African fields. This may be a major reason for the low per hectare yields in sub-Saharan Africa, compared with the high yields in South Asia where cassava is fertilized (<http://www.fao.org/3/I9004EN/i9004en.pdf>). Conversely, over-fertilization causes phenological changes that enhance shoot growth at the cost of root production (Pellet and El-Sharkawy, 1993). A better understanding of nitrogen metabolism in cassava is crucial to enhance root production, irrespective of whether this is done by improving agronomical practice, cultivar selection, or breeding.

Cassava is characterized by a high content of cyanogenic glucosides. Linamarin is the major cyanogenic glucoside in cassava, with significant amounts accumulating in all organs including leaves, stems, and roots (Picmanova *et al.*, 2015). Cyanogenic glucosides are considered to function in herbivore deterrence, as these compounds release highly toxic cyanide when they are mixed with β-glucosidases at the event of herbivore attack (Gleadow and Moller, 2014). For this reason, linamarin must be removed before dietary use by humans or as feed. Linamarin is also hypothesized to serve as carbon and nitrogen sinks as it is a conjugate of glucose and cyanide moieties. However, although a possible recycling pathway for linamarin has been indicated from metabolite profiling, evidence is lacking to support its functionality *in vivo* (Picmanova *et al.*, 2015). As well as linamarin metabolism, biofortification of vitamin content in cassava (Sayre *et al.*, 2011; Li *et al.*, 2015; Fudge *et al.*, 2017), and more recently studies on its starch structure (Bull *et al.*, 2018) have begun to appear. However, there is still no comprehensive picture of primary metabolism in cassava.

In order to identify which metabolic characteristics are related to yield traits in cassava, we grew six African cassava cultivars namely TMEB419, TMEB693, IITA-TMS-IBA980002, IITA-TMS-IBA980581, IITA-TMS-IBA30572, and IITA-TMS-IBA011412 under controlled well fertilized greenhouse

conditions in Erlangen, Germany and in the field at IITA in Ibadan, Nigeria. We harvested source leaves, sink leaves, upper stems and storage roots for metabolite and enzyme activity profiling, and determined yield-associated traits and performed pairwise correlation analyses between each metabolic trait and the yield-associated traits. We paid attention to differences between field and greenhouse conditions, as well as to intercultivar differences in specific metabolic traits. We also compared the metabolite levels and enzyme activities in cassava to those previously documented in other crops. These analyses are discussed within the context of the metabolic traits that underlie the high photosynthesis rates in cassava, the contribution of source and sink to root yield in different growth conditions, the response to nitrogen supply, the balance between central metabolism and aspects of specialized metabolism, and possibilities for improving root yield under limited input agriculture.

RESULTS

Six cultivars of cassava showed different root weights in both greenhouse and field trials

To investigate intercultivar differences in yield, storage root weight, shoot weight, plant total weight and the harvest index (root weight divided by total plant weight) were determined in six cassava genotypes after storage root bulking [12 and 16 weeks after planting (WAP), in greenhouse and field trials, respectively]. The greenhouse regime was characterized by higher N supply, and lower irradiance. These parameters were also determined after 1 year (52 WAP) in the field trial to assess final yield at agricultural harvest.

Root weight and harvest index were lower in the greenhouse than in the field (Figure 1, note scale differs between panels). There were also marked differences between the six cultivars. Generally, in a given condition, cultivars showed similar shoot weights but rather different root weights. For example, root weight was high in IITA-TMS-IBA980002 under greenhouse conditions, and in TMEB419 in the field at the bulking stage. These variations in the root weight were reflected in the harvest index. Interestingly, IITA-TMS-IBA980002 showed the highest harvest index in the greenhouse, but a low harvest index in the field at the bulking stage (Figure 1). As such, root weight at the early stage of storage root development varied among cultivars and environments. Five of the cultivars did not display dramatic differences in root weight in the field at agricultural harvest (TMEB419 was not included in this harvest).

Tissue-specific accumulation of metabolites are more prominent in the greenhouse-grown plants

Chemical properties including abundance of starch, protein, sugars, sugar phosphates, nucleotides, amino acids,

organic acids, and minerals in the source leaf, sink leaf, storage roots, and upper stem were determined 16 WAP in the field-grown plants and 12 WAP in greenhouse-grown plants. In total, 100 chemical properties were determined by multiple analytical approaches (Data S1).

Principal component analysis (PCA) showed clear separation of the samples from each tissue in both the field and the greenhouse (Figure 2a,c). The storage root was characterized by high starch content under both conditions (Figure S1). Ornithine and arginine contributed to the separation of roots from other tissues in the greenhouse samples (Figure 2b) but made only minor contributions in field material (Figure 2d). Glutamine and putrescine characterized the stem in the greenhouse-grown plants (Figure 2b). Putrescine made little contribution (Figure 2d) and glucose, fructose, and nitrate made more prominent contributions (Figures 2b,d, and S1) to separation of the stem samples in field-grown material.

The relative levels of metabolites normalized by the median of the contents of each metabolite are shown as a heatmap (Figure 3). Tissue-specific accumulation can be seen for metabolites belonging to the bottom cluster, which contained putrescine, glutamine, ornithine, arginine, asparagine, serine, proline, nitrate, and trehalose. However, tissue-specific accumulation of these metabolites was less evident in field-grown than greenhouse-grown material (Figure 3). This cluster included some major amino acids and their derivatives (which accumulated to above 1 mg g^{-1} in at least one of the tissues).

Tissue contents of selected metabolites are shown in the Figure 4 (the results for all metabolites are provided in the Data S2). As already mentioned, metabolites were often highly accumulated in one or two tissues in the greenhouse-grown plants, while tissue specificity was less evident in field-grown plants. Glutamine and putrescine reached 641 and 5 mg g^{-1} in the stem of greenhouse-grown plants, respectively. Alanine also displayed stem-specific accumulation but in this case the effect of growth environment was less evident. Arginine and ornithine accumulated in sink leaves and roots of greenhouse-grown plants. Asparagine, glutamic acid, and serine accumulated in sink leaves of greenhouse-grown plants. Aspartic acid also accumulated in the sink leaves of the greenhouse-grown plants while it accumulated in roots in the field-grown plants (Figure 4). The generally high levels of major amino acids in the greenhouse plants may reflect the higher N supply. In contrast, the minor amino acids in the bottom cluster in the Figure 3 showed no clear changes in tissue distribution between greenhouse and field trials and were unevenly accumulated among tissues (Figure S2). A few amino acids showed tissue-specific accumulation only in field-grown plants, including tryptophan, glycine and leucine that were highly accumulated in leaves of the field samples (Figure S3).

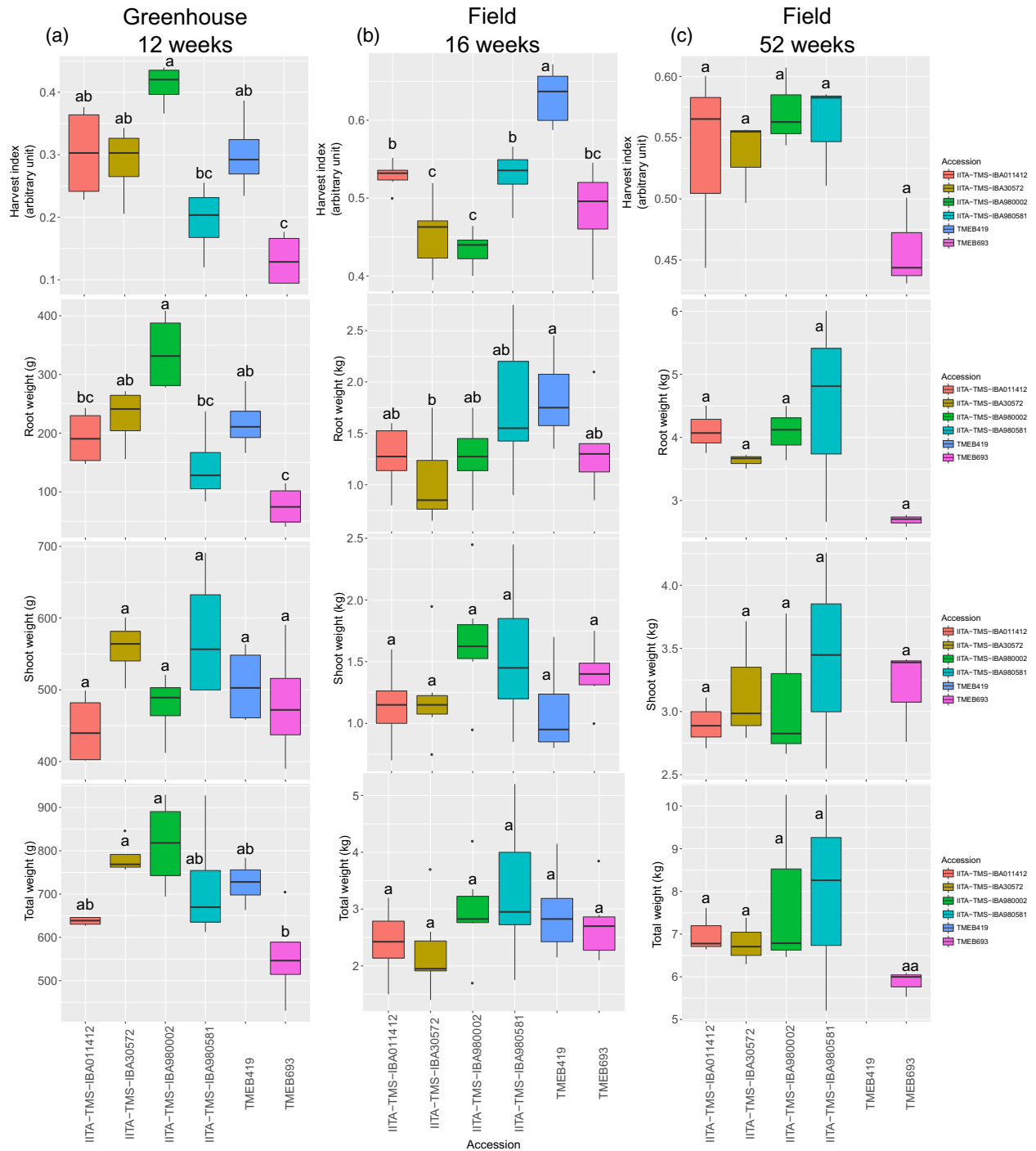


Figure 1. Agronomic traits of six commercial cassava cultivars in the greenhouse-grown plants at 12 weeks after planting (a) and field-grown plants at 16 weeks (b) or 52 weeks (c) after planting (note scale differs between panels). The boxplots showing the minimum, first quartile, median, third quartile, and maximum of harvest index, shoot weight, root weight and total plant weight are presented in the top to the bottom panels. One-way ANOVA was conducted to examine the variation in yield traits among cultivars and the letters indicate the results of following Tukey honestly significant difference (HSD) test comparing values among cultivars in the same condition ($P < 0.05$).

High contents of linamarin were detected in all tissues, although accumulation was less marked in root material (Figure 4). The linamarin content was similar in

greenhouse-grown and field-grown plants, except that the sink leaf content was slightly higher in greenhouse-grown plants (Figure 4). Source leaf-specific and stem-specific

Greenhouse

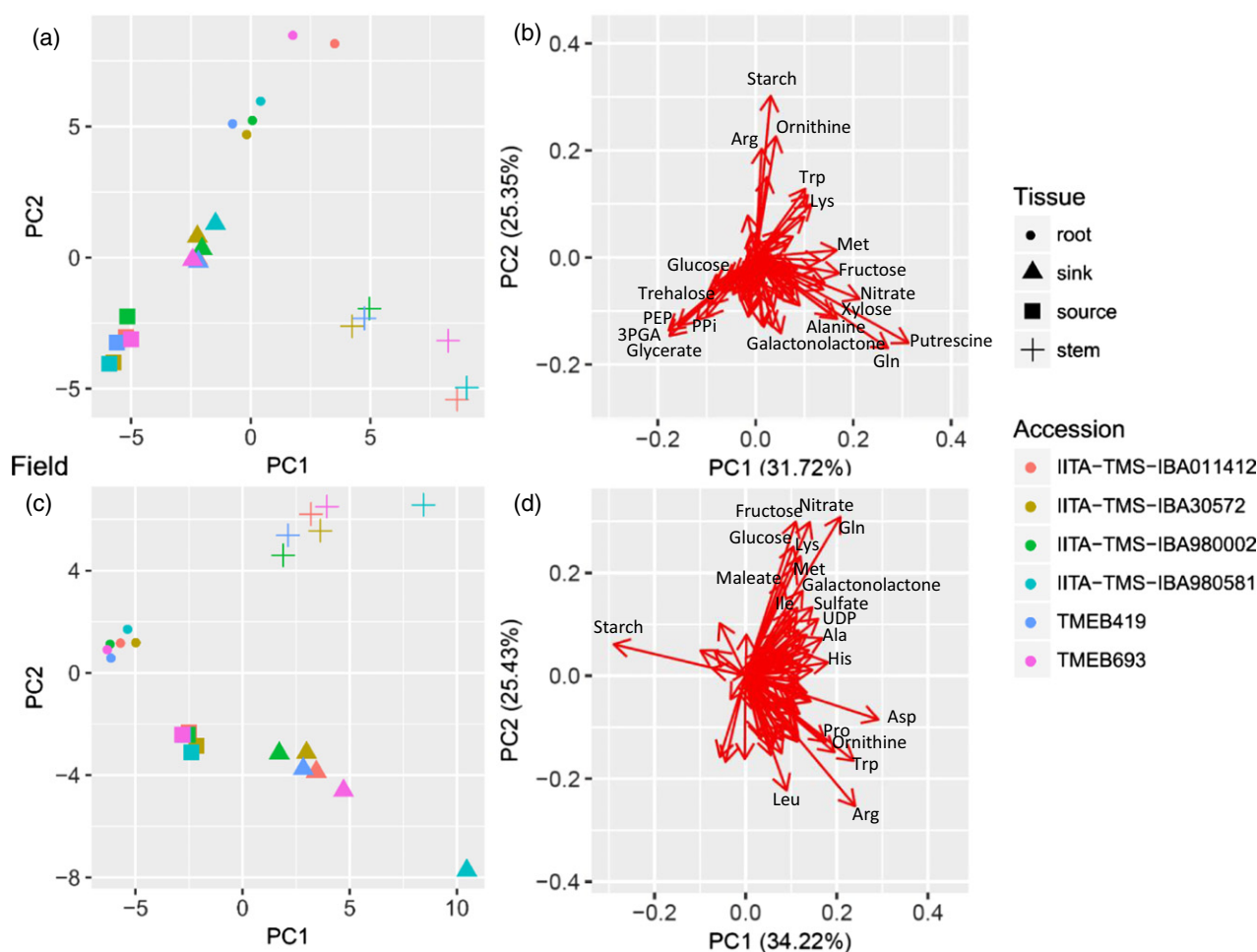


Figure 2. Differences in metabolite profiles among tissues from six cassava cultivars grown in greenhouse and field trials. Principal component analysis (PCA) of metabolic traits in the greenhouse (a) or field (c) experiments and respective loading plots including the names of 20 variables contributed most in the separation in PC1 and PC2 (b, d). The content of each metabolite was normalized by the median of that in all measurements and \log_2 transformed. The means of the values in each cultivar and tissue were analyzed.

accumulation of trehalose was found in greenhouse-grown but not in field-grown plants (Figure 4). Some organic acids and sugar monophosphates (Figure S4) occurred at higher levels in source leaves of greenhouse-grown than field-grown plants, whereas their levels in other tissues were similar under both growth environments. Roots accumulated much more starch than other tissues in both environments (Figure 4). Close inspection revealed that source and sink leaves accumulated more starch under field conditions than under greenhouse conditions. Sucrose contents in leaves and stem were comparable between greenhouse- and field-grown plants (Figure 4). However, whereas sucrose in storage roots was very low in field-grown plants, it was almost three times higher in the greenhouse plants (Figure 4). This may point to a restriction on sucrose use in the roots in the greenhouse plants. Most minerals accumulated in a similar manner in

greenhouse-grown and field-grown plants (Data S2). However, stem-specific accumulation of nitrate was much more prominent in greenhouse-grown plants (Figure 4).

Metabolite contents in leaves correlated significantly with root yield parameters

In order to identify metabolic traits that are potentially related to cassava yield, correlations between metabolite contents in each tissue and yield parameters were tested by Pearson correlation analysis. For the greenhouse trial, metabolites and yield traits were compared at bulking (12 WAP). For the field trial, metabolites at bulking (16 WAP) were compared with yield traits at bulking (16 WAP) and agricultural harvest (52 WAP). The results of all correlation tests are shown in Data S3, and genotypic variation of metabolite levels in each tissue is shown for all metabolites in Data S4.

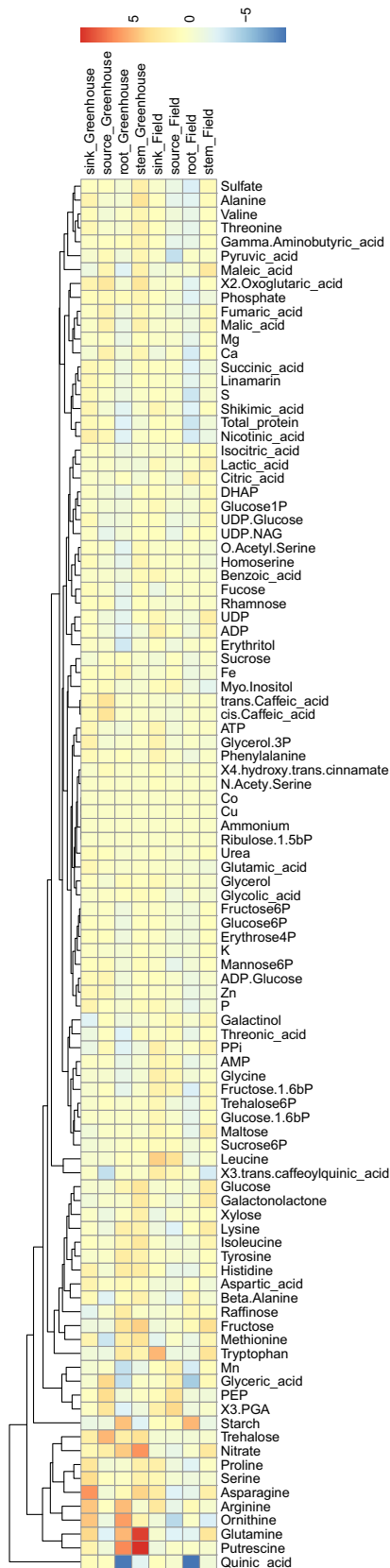


Figure 3. Heatmap of relative metabolite levels in each tissue in greenhouse and field trials. The content of each metabolite was normalized by the median of that in all measurements and log₂ transformed. The means of the values in each tissue from all cultivars were shown as a heatmap. Red and blue colours represent high and low levels of metabolites using a false-colour scale, respectively. Metabolites are arranged based on the result of hierarchical clustering and the dendrogram is shown on the left.

Under greenhouse conditions, eight metabolites in source leaves displayed significant correlations with yield traits (Figure 5a). Under field conditions, two metabolites in source leaves and two metabolites in sink leaves displayed significant correlations with yield traits (Figure 5b). In both conditions, we did not detect any significant correlations between metabolites in stem or storage roots and root yield traits. Sucrose and sugars showed minor variation among cultivars in both greenhouse and field environments (Figure S5).

In greenhouse-grown plants (Figure 5a), glutamic acid and Mg showed negative and positive correlations, respectively, with both harvest index and root weight, threonine and AMP showed negative correlations with harvest index, and leaf K correlated negatively with root yield. Genotypic variation of metabolite and ion contents in source leaves of greenhouse-grown plants is shown in the Figure 6a. Source leaves of TMEB693, which showed poor root yield in the greenhouse (Figure 1), had high levels of glutamate, threonine, AMP and K, all of which showed negative correlations with yield parameters (Figure 5a) and low levels of Mg, which showed a positive correlation with yield. Source leaves of IITA-TMS-IBA980002, which showed the highest yield performance (Figure 1), tended to accumulate less glutamate, AMP and K than other cultivars and the highest amount of Mg (Figure 6a).

In field-grown plants (Figure 5b), AMP in sink leaves showed a positive correlation with shoot weight at 52 WAP, 3-*trans*-cafferoylquinic acid (chlorogenic acid) in source leaves showed a positive correlation with harvest index at 16 WAP (Figure 5b). These positive correlations depended on the TMEB419 cultivar, which accumulated much higher levels of trehalose and chlorogenic acid in leaves (Figure 6b) and had a high yield (Figure 1b) in the field.

Enzyme activities reveal a high capacity for photosynthesis and sucrose and starch synthesis in leaves

We profiled the activity of a panel of enzymes involved in photosynthesis, starch and sucrose synthesis, nitrogen metabolism, glycolysis and respiratory metabolism using assays previously established for Arabidopsis (Gibon *et al.*, 2004; Sulpice *et al.*, 2007), tomato fruit (Steinhauser *et al.*, 2010) and maize leaves (Wang *et al.*, 2014; Table S2). For

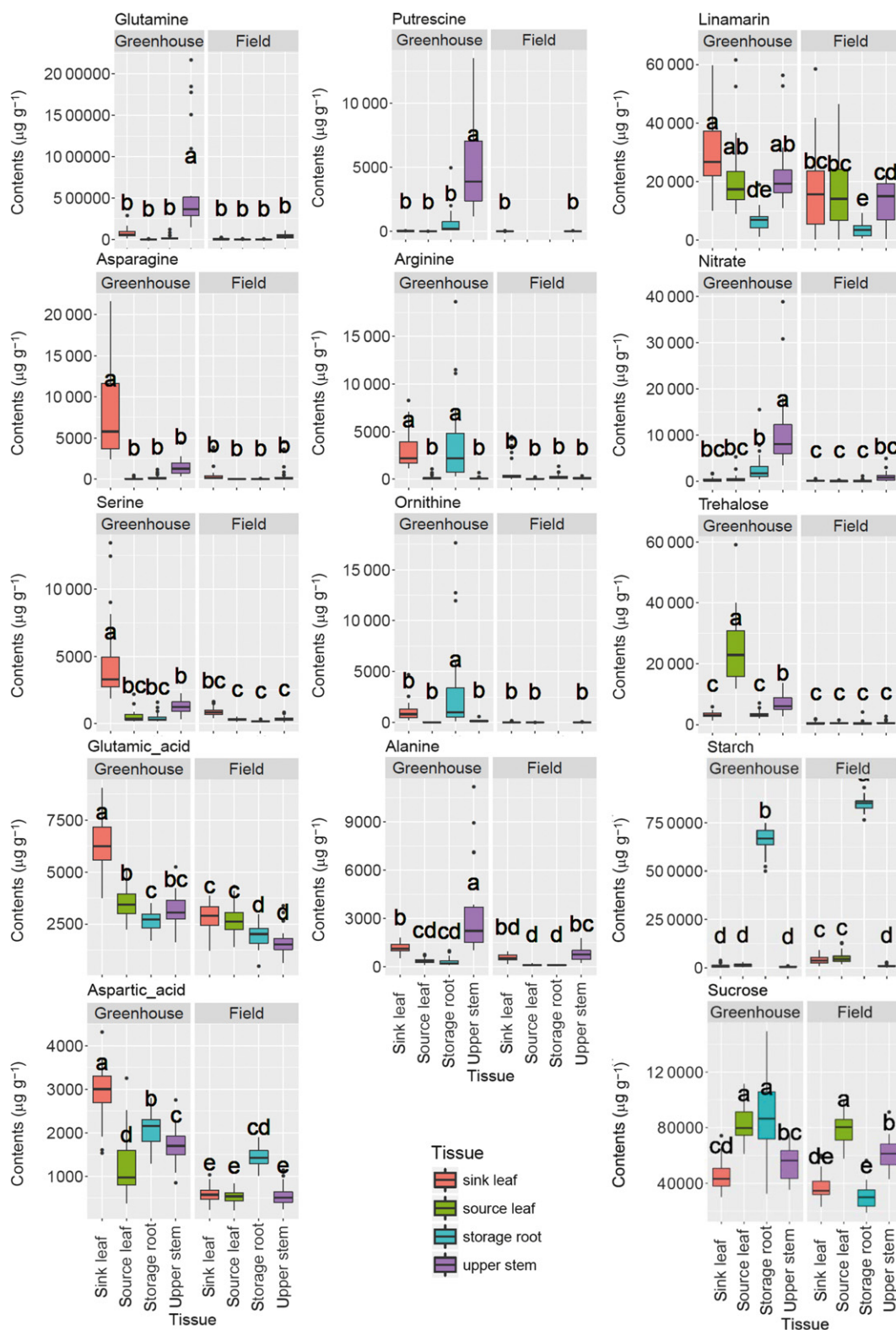
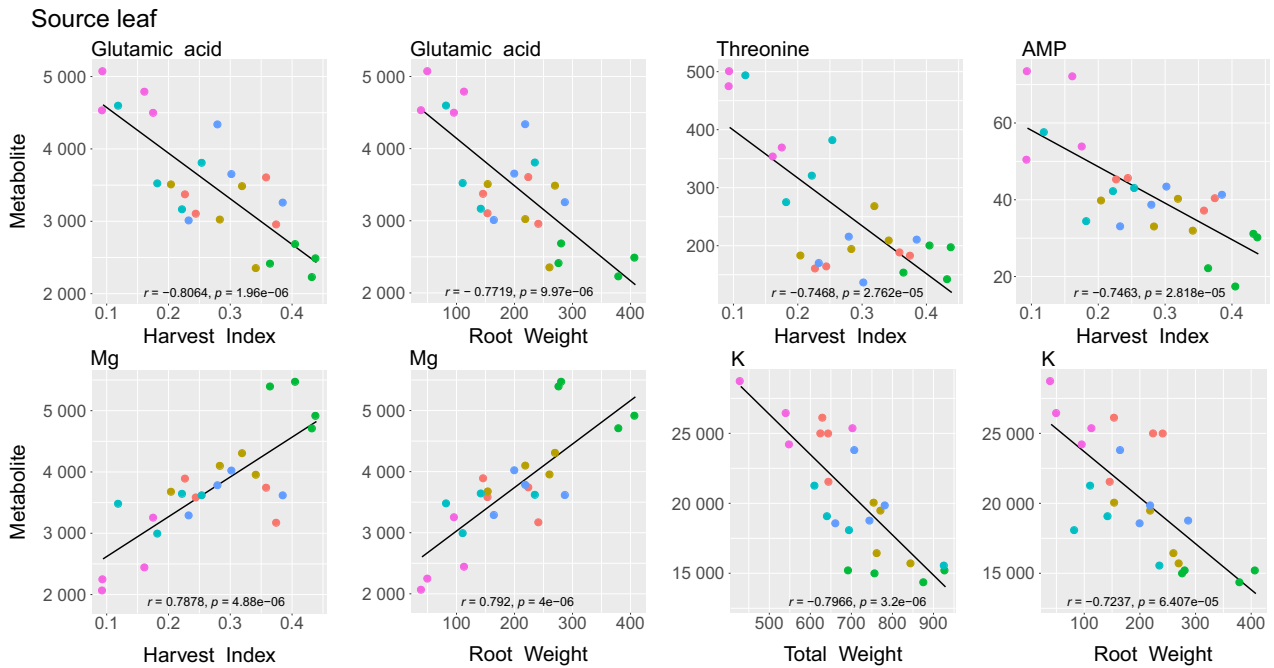


Figure 4. Contents of metabolites which showed remarkable tissue specificity in greenhouse and field-grown cassava plants. The boxplots show the minimum, first quartile, median, third quartile, and maximum contents of metabolites in tissues from each of four or six plants of all six cultivars in the greenhouse and the field experiments, respectively ($n = 24$ greenhouse, $n = 36$ field). The left and right panels represent the results from greenhouse and field experiments, respectively. One-way anova was conducted to examine the variation in metabolite contents among tissues and the letters indicate the results of following Tukey honestly significant difference (HSD) test comparing values among tissues under the same conditions ($P < 0.05$).

(a) Greenhouse



(b) Field

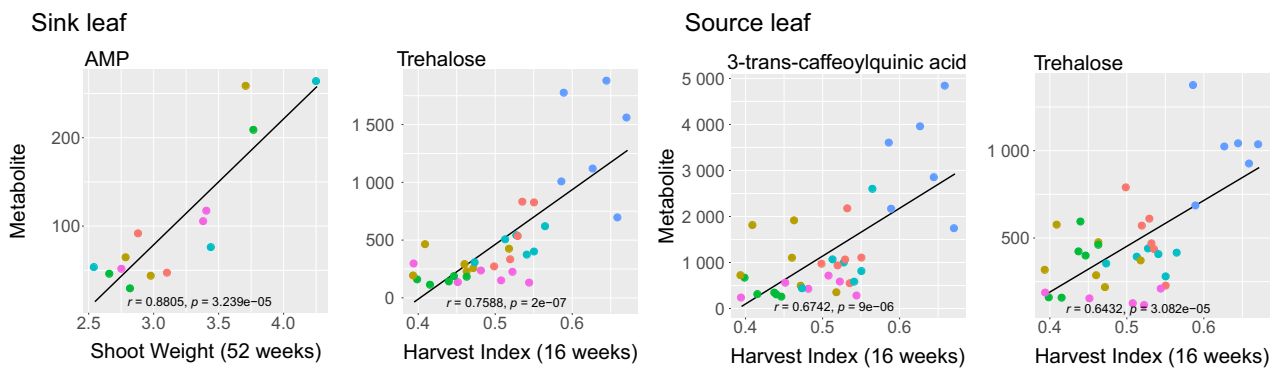


Figure 5. Correlations between metabolite contents in leaf tissues and yield parameters of greenhouse-grown (a) and field-grown (b) cassava plants. Contents of selected metabolites showing significant correlation with yield parameters ($P < 3.125 \times 10^{-5}$; Data S3) were plotted against harvest index or root weight. Each point indicates the data from an individual plant ($n = 24$: 4 plants \times 6 cultivars in the greenhouse; $n = 36$: 6 plants \times 6 cultivars in the field). Colours of the points correspond to cultivars. r and P are the correlation coefficient and P -value from Pearson correlation analysis, respectively.

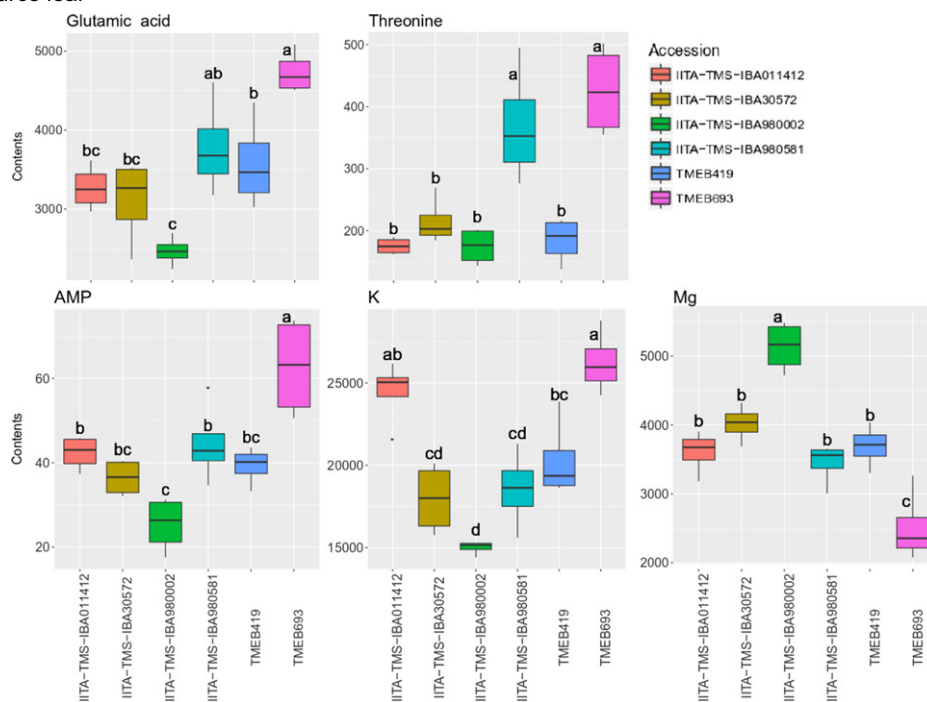
each enzyme and tissue, assays were optimized by modifying pH, ion and substrate levels to achieve maximal activity, and validated by checking for linearity of activity with extract amount (Tables S3 and S4). In all analysis batches, we included standard greenhouse-grown *Arabidopsis* rosette material as a control. Enzyme activities were analyzed in the greenhouse trial in Erlangen and two field trials at IITA in 2015 and 2016, with the latter being a dry year (data provided in Data S5). Whereas enzyme activities could be harvested in fresh material from the greenhouse, transport of field-grown material required lyophilization. This led to loss of activity for some enzymes, which were therefore excluded from the data set for field-grown material.

We first compared enzyme activities between genotypes. Variation was larger between trials than between genotypes, as illustrated by selected enzymes in the mature leaf (Figure S6a) and storage root (Figure S6b) of the six genotypes that were jointly investigated in the greenhouse and one of the field trials. In a PCA, samples were grouped according to trial, rather than to genotype (Figure S6c).

We therefore focused on features that were shared between genotypes. One striking result was that the activities of enzymes related to photosynthesis and starch and sucrose synthesis were very high in cassava source leaves. Figure 7 shows enzyme activities for greenhouse-grown plants. Enzymes that were especially high in cassava relative to *Arabidopsis* included photosynthesis enzymes

(a) Greenhouse

Source leaf



(b) Field

Sink leaf

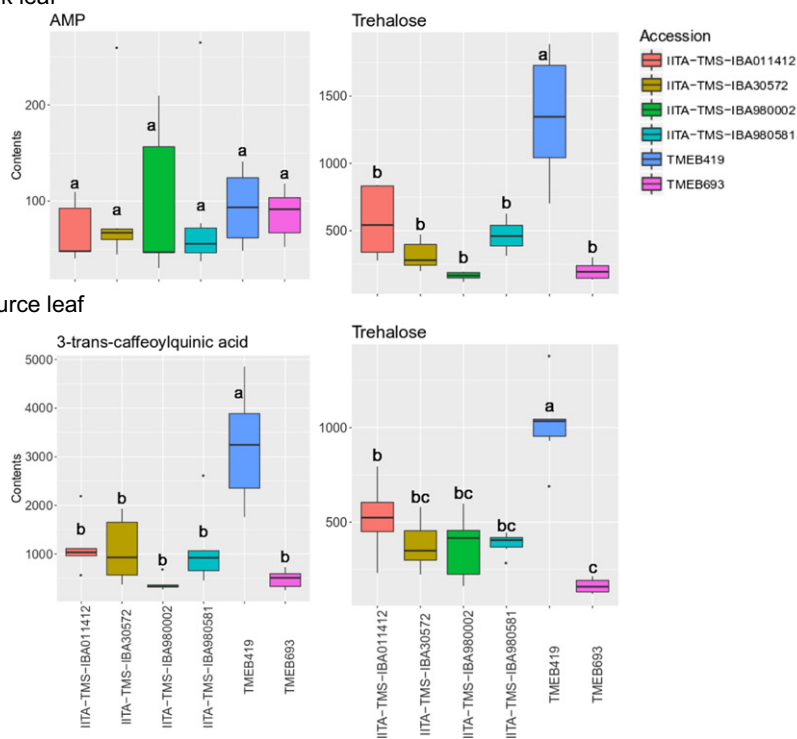


Figure 6. Genotypic variation of metabolite contents in source leaves of greenhouse-grown (a) and field-grown (b) cassava plants. Contents of metabolites in tissues which showed significant correlation with yield parameters (Figure 5) are shown. The boxplots show the minimum, first quartile, median, third quartile, and maximum contents of metabolites in each cultivar ($n = 4$ greenhouse, $n = 6$ field). One-way ANOVA was conducted to examine the variation in metabolite contents among cultivars and the letters indicate the results of Tukey honestly significant difference (HSD) test comparing values among cultivars ($P < 0.05$).

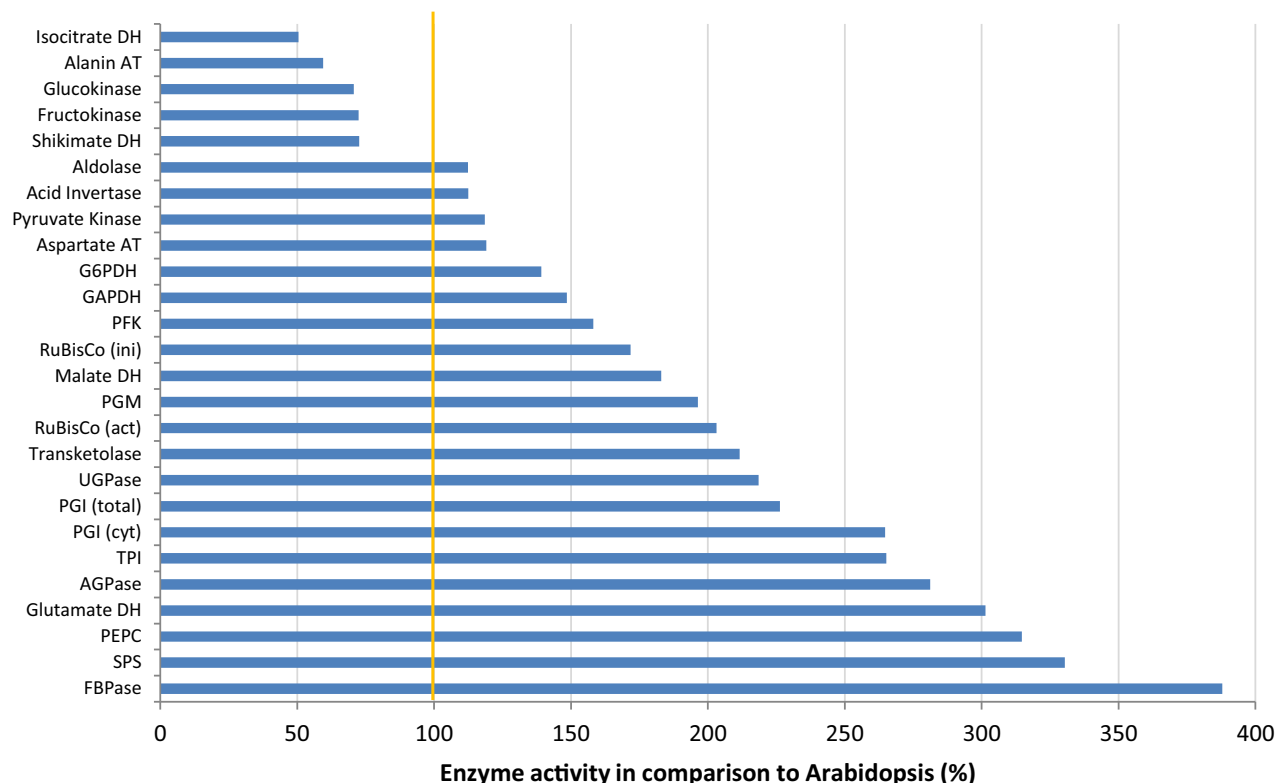


Figure 7. Enzyme activities in mature cassava leaves from the cultivar TME419/TMEB419 compared with *Arabidopsis* rosettes. Both sets of plants were grown in the greenhouse. The original data are provided in Table S6. Similarly high activities of enzymes from the Calvin–Benson cycle, starch and sucrose synthesis and phosphoenolpyruvate carboxylase (PEPC) were also seen in the other cultures and, for enzymes that could be measured, in the field-grown plants (Data S5 and Figure S6).

(FBPase, RuBisCO, TK, TPI), enzymes of sucrose and starch synthesis (AGPase, SPS, UGPase) and PEP carboxylase. Those enzymes that could be measured in lyophilized material had even higher activity in field-grown than greenhouse material (Figure S6a). The high activities of photosynthesis-related enzymes in cassava leaves may partly reflect their high protein and chlorophyll content (Data S5; Arrivault *et al.*, 2019). Conversely, many enzymes that are involved exclusively in respiratory metabolism [G6PDH, pyruvate kinase, fructokinase, glucokinase, isocitrate dehydrogenase (DH)] and N metabolism (AspAT, AlaAT) were similar or lower in cassava than *Arabidopsis*.

The relatively high PEP carboxylase activity in cassava leaves prompted us to directly compare RuBisCO and PEP carboxylase activities in cassava, the C_3 species *Arabidopsis* and the C_4 species maize, all measured on the same analytic platform (Table S5). The high PEP carboxylase activity in cassava largely paralleled the high RuBisCO activity. The ratio of PEP carboxylase activity:RuBisCO activity was low in cassava and *Arabidopsis* (0.31 and 0.17, respectively) and 15–40 times higher in maize (3.3–8.1).

AGPase activity in roots was consistently about two-fold higher in field-grown than greenhouse-grown material

(Table S6 and Figure S6b). This contrasts with the glycolytic enzymes phosphofructokinase (PFK) and pyruvate kinase (PK), which showed similar activities in field and greenhouse material. This high AGPase activity may partly explain the higher starch and lower sucrose contents in roots from field-grown compared with greenhouse-grown plants (see above). AGPase, PFK, and PK activities were all higher in the stems of field-grown than greenhouse-grown plants (Table S6), possibly reflecting an increased importance of the latter as a transient store and metabolic compartment in the field.

DISCUSSION

High activities of enzymes involved in photosynthesis and sucrose and starch synthesis in cassava leaves support high photosynthesis rates

According to FAO statistics, cassava is currently the fourth most important crop on Earth in terms of calorific yield (<http://www.fao.org/publications/sofa/2016/en/>). However, fundamental metabolic features pertinent to yield remain under-researched. One striking feature of cassava metabolism uncovered in this study is the very high activities of

enzymes involved in photosynthesis, sucrose synthesis and starch synthesis in source leaves (Figure 7). The Calvin–Benson cycle enzymes investigated in this study included RuBisCO, FBPase and TK; RuBisCO and FBPase often exert a considerable and a small amount of control on the rate of photosynthesis, respectively, and TK is only in small excess (Henkes *et al.*, 2001; Tamoi *et al.*, 2006; Stitt *et al.*, 2010; De Souza *et al.*, 2019). These high enzyme activities may contribute to the high rates of photosynthesis in cassava (Angelov *et al.*, 1993) and to the high sucrose levels in its source leaves, compared with other crops. The relatively high activity of PEP carboxylase may contribute to increased synthesis of organic acids and amino acids.

Root yield is correlated with several metabolic traits in the leaves

All of the metabolic traits that showed significant correlation with yield traits were for leaves, and most were for source leaves (Figure 5). Metabolite contents in storage roots did not show any significant correlations with yield traits in the greenhouse or field trial. This situation is most likely due to the increase in storage root mass keeping the root metabolite content constant on a dry weight basis. It is rather reasonable for plants to extend the storage capacity by increasing storage root mass rather than accumulating metabolites in the roots. The preponderance of source leaf metabolic traits in the correlation matrix between metabolic and yield traits indicated a close relationship between source metabolism and root yield in cassava, especially in greenhouse plants. However, we did not find any obvious relationship of yield traits with sugar or starch contents in the source leaf.

The yield traits in greenhouse plants positively correlated with Mg and negatively with glutamate, threonine and AMP, while those in field-grown plants correlated positively with trehalose, 3-*trans*-caffeoylquinic acid (chlorogenic acid) and AMP (Figure 5). Some of these relationships point to a relationship between energy status and yield. Mg has been reported to have a positive impact on the yield in many crops. It has various functions related to photosynthesis including being required for chlorophyll and RuBisCO activity, chloroplast ultrastructure and phloem loading and photoassimilate transport (Trankner *et al.*, 2018). AMP may activate SnRK1, which plays a central role in the regulation of metabolism in response to energy and stress (Crozet *et al.*, 2014). Trehalose provides protection against abiotic stress. It is also associated with the trehalose-6-phosphate (T6P) signalling pathway, which regulates carbon and nitrogen metabolism, as well as developmental transitions (Lunn *et al.*, 2014; Figueroa and Lunn, 2016), and acts at least in part by inhibiting SnRK1 (Zhang *et al.*, 2009; Debast *et al.*, 2011; Zhai *et al.*, 2018). However, the signalling function of trehalose metabolism

in cassava has not been investigated and sucrose levels showed no correlation with yield parameters.

Source leaf K correlated negatively with root and total weight in greenhouse-grown plants (Figure 5). This is unexpected because K usually has positive effects on photosynthesis, phloem transport and crop yield (Trankner *et al.*, 2018) and K deficiency impacts on organic acid and nitrogen metabolism (Armengaud *et al.*, 2009). Although cassava absorbs large amounts of K from the soil, K often becomes the most deficient nutrient and can restrict root production (El-Sharkawy, 2012). The negative correlation between K and yield traits might reflect an interaction between K and sucrose transport from source leaves to storage roots. K is a major solute in sieve cells (Mengel and Haeder, 1977; Prajapati, 2012) and sucrose loading depends on a concomitant import of K (Giaquinta, 1980). The negative correlation between source leaf K and root weight might indicate that transport of sucrose to sink tissues is supported by rapid K⁺ loading into the phloem, which reduces source leaf K levels. A major function of K import into the phloem is to reload the ‘potassium battery’ (Gajdanowicz *et al.*, 2011). The phloem is prone to hypoxia, leading to decreased energy charge (van Dongen *et al.*, 2003) and this is especially likely in plants with a thick stem, like cassava. A low energy charge and accompanying membrane depolarization usually lead to substrate leakage (Minchin and Thorpe, 1987), for which high concentrations of K in the phloem serve to counteract.

Cassava yield is mainly source limited and sink limited under greenhouse and field conditions, respectively

The contribution of source and sink limitation depends on conditions and development (Sonnewald and Fernie, 2018). The growth conditions in the field and the greenhouse environments of our study differed, with lower light intensity and temperature but higher nitrogen availability in the greenhouse than in the field. This is likely to lead to stronger source limitation in the greenhouse compared with the field-grown plants. The preponderance of correlations between source leaf metabolite levels and root yield parameters in the greenhouse condition (Figure 5) is consistent with the idea that source metabolism limits the root yield. This conclusion is in agreement with the observation that cassava tuber yield is increased under elevated atmospheric CO₂ (Rosenthal *et al.*, 2012).

De Souza and Long (2018) suggested that cassava can become sink limited in conditions that allow high rates of photosynthesis. In our study, field-grown plants had a much higher root yield than greenhouse plants. This was accompanied by a two- to three-fold increase in AGPase activity (Figure S6b) and a three-fold decrease in sucrose levels (Figure 4) in the root. AGPase typically exerts control over starch synthesis, especially in sink organs (Geigenberger *et al.*, 2004; Smidansky *et al.*, 2007; Zeeman *et al.*,

2010). Our results indicated that under greenhouse conditions the conversion of sucrose to starch in the roots may be restricted by low AGPase activity, and that this restriction is at least partially relieved in field-grown plants. As already mentioned, the rate of photosynthesis and the supply of sucrose to the roots is likely to be higher in the field-grown plants than in greenhouse plants. The increase in AGPase activity may be important to support the higher flux to starch in roots. Further, by reducing root sucrose levels it may promote movement of sucrose to the root. It is possible that the increase in AGPase activity is accompanied by co-ordinated increase in the activity of other enzymes or transporters involved in sucrose breakdown but, notably, it was not accompanied by an increase in the activity of the key respiratory enzymes PFK and PK. Further experiments are needed to identify the molecular mechanisms that lead to the increase in AGPase activity in the field, but they are likely to include positive effects of carbon signalling and possibly negative effects of nitrogen signalling (Scheible *et al.*, 1997; Nielsen *et al.*, 1998; Stitt *et al.*, 2002) on AGPase expression (see also below for a wider discussion of the impact of nitrogen signalling on storage roots). Although not addressed in our study, sugars also promote post-translational activation of AGPase (Tiessen *et al.*, 2002; Geigenberger *et al.*, 2004). Taken together, our results pointed to a shift towards sink limitation in the field, albeit alleviated by feedforward regulation of AGPase and possibly further steps in the conversion of sucrose to starch. Further evidence for a key role of AGPase in starch accumulation in cassava roots is provided by a study in which the bacterial *glgC* gene (a deregulated version of the starch biosynthesis enzyme AGPase) was overexpressed in cassava storage roots (Ihemere *et al.*, 2006). Compared with control plants, this resulted in a 2.6-fold higher storage root biomass and significant increases in above-ground biomass consistent with a possible reduction in feedback inhibition of photosynthetic carbon fixation. This earlier finding highlights the importance of the difference in AGPase activity that we observed between greenhouse and field conditions.

Taken together, cassava root yield is likely to be co-limited by source and sink, as has previously been documented for potato tuber yield (Sweetlove *et al.*, 1998; Jonik *et al.*, 2012; Sweetlove *et al.*, 2017). In our greenhouse plants, the supply of carbon is likely to be low, AGPase activity is low, and root yield is low. In field-grown plants, the supply carbon is likely to be higher, AGPase activity is increased and root yield is higher. This close interaction between source and sink capacities implies that optimal yield improvement of African cassava is likely to require intervention in both source and sink functions. At first sight, this conclusion is unexpected, as cassava has very high rates of photosynthesis for a C₃ species (see above). However, photosynthesis rates including cumulative

photosynthesis over a season are not always high, and photosynthetic efficiency is only *c.* 14% of the theoretical maximum for a C₃ plant (De Souza *et al.*, 2017). Furthermore, conversion of photosynthate into tuber biomass is rather inefficient due to simultaneous growth of the shoot, stem and tubers (De Souza *et al.*, 2017).

Yield is not strongly limited by nitrogen

Many major metabolic characteristics of the various tissues in cassava were similar between the two growth conditions despite vast differences in the parameters including light and temperature regimes (Figures 3, 4, and S1–S5). In both greenhouse-grown and field-grown plants, the storage root was characterized by high starch and low protein, while the stem was characterized by high levels of glucose, fructose and glutamine and high linamarin (Figures 2 and 4). A different picture appears for nitrogen-containing primary metabolites, with most of these being considerably higher in greenhouse-grown than field-grown plants (Figure 4). This situation is almost certainly due to the paucity of soil nitrogen in Ibadan with total nitrogen representing 0.07 % of soil mass in Ibadan, compared with 0.12% in the soil used in the greenhouse (Table S7). Differing nitrogen supply may also explain the lack of consistency between yield data in the greenhouse and the field (Figure 1). As mentioned in the Introduction, over-fertilization of cassava can enhance shoot growth at the cost of root production (Pellet and El-Sharkawy, 1993). This may explain why all the cultivars we tested, except for IITA-TMS-IBA980002, showed lower harvest index in the greenhouse than in the field. The inconsistent root yield in the two growth environments may reflect cultivar-specific sensitivities of growth to nitrogen availability. For example, our results suggested that IITA-TMS-IBA980002 may be tolerant to excess nitrogen. These will in turn affect the carbon source–sink interactions (Burnett *et al.*, 2016). It is also possible that further factors like light and temperature, a less stable environment and biotic interactions contribute to the different responses in the greenhouse and field.

Our study provides new insights into nitrogen metabolism in cassava. First, total protein content in cassava, surprisingly, showed only minor differences between the greenhouse and the nutrient-impooverished field (Figure S1), despite quantitative differences in the levels of many abundant amino acids (Figure 4). Photosynthesis enzymes represent a large part of total leaf nitrogen. As already mentioned, their activities were even higher in field-grown than greenhouse-grown material, pointing to the ability of cassava to maintain photosynthesis capacity under low nitrogen conditions. Second, increased tuber yield in the field compared with the greenhouse was associated with lower amino acid levels in many organs (Figures 4 and S2). This might reflect a restriction of tuber initiation or growth by high nitrogen (Pellet

and El-Sharkawy, 1993). This is also indicated by the negative correlation between yield traits and source leaf glutamate and threonine contents, which was observed specifically in the greenhouse-grown plants (Figure 5). Third, in the fertilized greenhouse-grown plants; a subset of N-containing metabolites accumulated to very high levels, including glutamine, putrescine and nitrate in the stem, and ornithine and arginine in roots and sink leaves (Figure 4). Asparagine, serine and glutamate showed similar trends in sink leaves in greenhouse-grown and field-grown plants (Figure 4). These amino acids are likely to serve as nitrogen storage and for transport (Nordin and Nasholm, 1997; Couturier *et al.*, 2010). It is also noteworthy that the cyanogen linamarin, which contains nitrogen and is highly abundant in aerial tissues, showed higher accumulation in greenhouse than in field material (Figure 4) suggesting that linamarin probably serves as a nitrogen storage compound. Linamarin is considered a nitrogen storage molecule (Sirtunga and Sayre, 2004) as it accumulates in the shoot apex when cassava is fertilized with nitrate (Jorgensen *et al.*, 2005) and the nitrogen in the cyanide moiety is incorporated into amino acids in cassava seedlings (Nartey, 1969). Although this cyanogenic glycoside accumulated to higher levels in the nitrogen-rich greenhouse conditions than in the field, the increase in amount is less than that of glutamine (Figure 4). Considering the high atomic N/C ratio in the molecules, glutamine and putrescine most likely serve as the major nitrogen storage in cassava. These results indicated distinctive mechanisms to maintain nitrogen homeostasis in cassava, which enables this plant to maintain the protein concentration in leaves, regardless of nitrogen availability and further its productivity on low nitrogen soil.

Comparative analysis of the absolute levels of metabolites across source and sink tissues with those of other better characterized crops such as potato (Roessner *et al.*, 2000; Fernie *et al.*, 2002; Evers *et al.*, 2010), and tomato (Roessner-Tunali, 2003; Schauer *et al.*, 2005; Leyva *et al.*, 2014; Benard *et al.*, 2015) revealed similar trends and similar ranges of absolute levels of most nitrogen-rich metabolites across all these species (Data S1). However, asparagine and glutamine were considerably higher in cassava than tomato leaves, while tryptophan, lysine and histidine were considerably lower (Data S1). Furthermore, tryptophan, lysine and histidine, were considerably lower in cassava roots than potato tubers or tomato fruits (Data S1). Interestingly, the amino acids that are higher in cassava contain more than one nitrogen atom. This comparison highlights its distinctive nitrogen metabolism and could contribute to the ability of cassava to maintain productivity on depleted soils. Our results also indicated that the stem metabolite profile is probably a good indicator of cassava nitrogen status, with putrescine accumulation in the stem potentially being a good marker of nitrogen soil nutrition as it was

undetectable in field-grown and high in greenhouse material.

Chlorogenic acid as a possible yield marker

We detected a few secondary metabolites with 3-*trans*-caffeoylquinic acid (chlorogenic acid), accumulating to much higher levels in TMEB419 than the other cultivars (Figures 5 and 6). This cultivar also showed the highest root weight and harvest index in the field (Figure 1). Chlorogenic acid functions as an antioxidant as well as an anti-fungal and anti-bacterial agent. It is likely that the capability of TMEB419 to accumulate chlorogenic acid in photosynthetic tissues renders it more resistant to pathogen attack, leading to the higher yield in the field. The low chlorogenic acid in the greenhouse may be because it is induced by pathogens that were absent in the greenhouse, or because chlorogenic acid is induced by UV-B (Tohge and Fernie, 2017). The ability to accumulate chlorogenic acid appears to be cultivar specific, making it a promising candidate marker to screen high yield cultivars and a potential breeding target to introduce environmental resilience to susceptible cultivars in the actual agricultural environment in Africa, along with trehalose which showed similar correlation and cultivar specificity (Figures 5 and 6).

In summary, profiling of metabolite levels and enzyme activities has provided new insights into metabolism in cassava. First, in addition to providing further support for the recent conclusion that cassava employs a C₃ mode of photosynthesis, our results revealed that the high rate of photosynthesis in cassava is likely to be due to high activities of photosynthetic enzymes. Second, storage root formation in cassava is likely to be co-limited by both sink and source capacity, with the interaction with the growth conditions. Third, yield is markedly robust against low nitrogen in the field, despite a decrease in the levels of nitrogen metabolites. This may be due to change in nitrogen metabolism compared with temperate crops. Glutamine and putrescine accumulated in the stem under the nitrogen-rich environment, most probably serving as forms of nitrogen storage that may partially support the high nitrogen-use efficiency of cassava plants. More generally, our dataset represents a valuable knowledge base for the analysis of cassava metabolism, for example absolute quantification of metabolite contents in each tissue provides information about their relative contribution in nitrogen and carbon metabolism. Our study also highlighted photosynthetic carbon assimilation rate (source capacity) under photosynthetically unfavourable conditions, K battery, regulation of root AGPase activity (sink capacity), and accumulation of chlorogenic acid as specific target traits for future improvement of this important crop species.

EXPERIMENTAL PROCEDURES

Materials and growth conditions

Six African commercial and farmer preferred cassava cultivars, TMEB419, TMEB693, IITA-TMS-IBA980002, IITA-TMS-IBA980581, IITA-TMS-IBA30572 and IITA-TMS-IBA011412, were grown in a greenhouse in Erlangen, Germany (August 2015 to October 2015) and in one experimental field in Ibadan, Nigeria (May 2015 to August 2016). The genotypes were chosen to cover diversity in yield, flowering time, resistance to cassava mosaic virus and other specific features. The information was extracted from historical data at IITA hosted by cassavabase.org. The characteristics of each genotype are described in Table S1. In the greenhouse experiment, plants from tissue culture were carefully transferred to substrate (Einheits Erde Classic Profi, Einheits Erde, Sinntal-Altengronau, Germany) in a 3.5-L pot and grown in a 20 m² chamber from 4 August 2015 until 29 October 2015. Growth light was supplemented with 250 μmol m⁻² sec⁻¹ under a 12 h/12 h, day/night light regime. The temperature was kept at 28 and 25°C in the day and night, respectively, and the relative humidity was kept at 60%. The plants were watered every second day and no fertilizer was supplied. Four individual plants of each cultivar were randomly located in the greenhouse, avoiding having the same genotype clustered together. The positions of these plants were rotated twice a week. In the 2015–2016 field trial, plants were planted on 28 May 2015. Sampling took place in the week of the 21 September 2015, when it was still in the high period of the first rainy season, to avoid metabolic and physiological changes caused by drought. The layout was a Randomized Complete Block Design in which three blocks (replicates) each containing in a randomized manner the same six genotypes in plots of 28 plants. The plants were planted as sticks (25 cm pieces from stem cuttings) from IITA internal genetic resource fields (genotyped by the cassava breeding unit). A similar field trial was conducted in July 2016 to July 2017. The material from 2016–2017 field trial was used only for enzyme activity measurements. The experimental design was the same as that of the 2015–2016 trial except that two genotypes, IITA-TMS-IBA980002 and IITA-TMS-IBA011412, were replaced with IITA-TMS-IBA980505 and IITA-TMS-EB7. A summary of experimental details, conditions and analysis performed is presented in Table S8.

Determination of agronomic traits

Plants were harvested for yield-associated traits at 12 WAP for greenhouse and at 16 and 52 WAP for field-grown plants. Each plant was carefully manually uprooted. Different plant parts were separated into storage roots, lignified stems, and young stem together with leaves. Different part weights were measured. For the greenhouse experiment, individual plants are treated as replicates. For the field sampling at 16 and 52 WAP, two individual plants per plot or pool of all plants (18–24 plants) from each plot were treated as replicates, respectively. The results are expressed in t/ha (10 000 plants per hectare, planted in a 1 m × 1 m grid).

Sample preparation for chemical profiling of cassava tissues

For detailed metabolic analysis, source leaves, sink leaves, upper stems, and storage roots were sampled at 12 and 16 WAP in the greenhouse and field trials, respectively. The materials were sampled from the plants used for the determination of agronomic traits. Sampling took place between late morning and early afternoon to minimize diurnal deviation in metabolite levels. Roots

were carefully washed before sampling. Samples from each tissue were snap frozen in liquid nitrogen immediately after sampling and freeze dried before shipments. The samples were ground well to a fine powder to be mixed well using a motor and pestle; aliquots were distributed to institutions for specific analyses.

Determination of organic acids and phosphorylated intermediates

Measurements of organic acids and phosphorylated intermediates were performed on 25 mg freeze-dried cassava tissue according to Horst *et al.* (2010a, 2010b). The recovery rate for each metabolite and tissue was determined before analysis by adding known quantities of authentic standards at the start of the metabolite determination process, that is extraction of the metabolites (Figure S7).

Determination of anions and elements

For the isolation of anions, 0.5 ml of water was added to 5 mg freeze dried and fine milled (Retsch, Haan, Germany) cassava tissue material, mixed thoroughly and heated for 15 min at 95°C. After centrifugation (10 min, 16 000 g), the supernatant was used for quantification via ion chromatography using a 761-IC compact device (Metrohm). The column system consisted of a Metrosep A Supp 4/5 Guard 4.0 and a Metrosep A Supp 4-250/4.0 (Metrohm), followed by conductometry at a flow rate of 1 ml min⁻¹ and a pressure of 7.5 MPa, using 1.8 mM sodium carbonate and 1.7 mM sodium hydrogencarbonate as the mobile phase and 50 mM sulfuric acid as the counter-ion.

The quantification of elements was performed as described earlier in Klaumann *et al.* (2011), with 20–30 mg of freeze-dried cassava tissue material. In brief, samples were placed in a mixture consisting of 5 ml HNO₃ (65%, v/v), 2 ml H₂O₂ (30%, v/v) and 3 ml double-distilled water in a MLS-Ethos microwave oven using a temperature step gradient with a maximum temperature of 210°C. Digests were analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES) on an iCAP 6300 DUO.

Determination of total protein

Total protein contents were quantified in freeze-dried cassava tissue material via the assay described in Bradford (1976) adapted for microplates.

Metabolic profiling

Metabolite extraction for gas chromatography-mass spectrometry (GC-MS) was carried out as previously described by Liseč *et al.* (2006) using 10 mg of ground freeze-dried cassava tissue. Ribitol (0.2 mg ml⁻¹ in H₂O) was used as an internal standard during extraction. A dry aliquot of the polar phase was resuspended in methoxyamine hydrochloride (20 mg ml⁻¹ in pyridine) and derivatized using *N*-methyl-*N*-[trimethylsilyl]trifluoroacetamide (MSTFA) containing 20 μl ml⁻¹ fatty acid methyl esters mixture as retention time standards. A volume of 1 μl of derivatized metabolite solution was used for injection. Peaks were annotated to metabolites by comparing mass spectra and GC retention times to database entries and those of authentic standards available in a reference library from the Golm Metabolome Database (Kopka *et al.*, 2005). The GC-MS system comprised a CTC CombiPAL autosampler, an Agilent 6890N gas chromatograph, and a LECO Pegasus III TOF-MS running in EI+ mode. Chromatograms and mass spectra were evaluated using Chroma TOF 1.0 (Leco) and TAGFINDER 4.0 software (Luedemann *et al.*, 2008). Mixtures of authentic standards with 0, 200, 500, and 1000 ng of each compound were included in each

batch of GC-MS analysis to calculate absolute amounts of metabolites from the peak intensity of a representative fragment.

The effects of freeze drying in the detection of individual metabolites in each tissue were determined before analysis. In total, 54 out of 59 detected metabolites showed less than 20% differences of peak intensities between fresh and dry samples (Figure S8), suggesting the minor effects of freeze drying on GC-MS quantification in general.

In order to evaluate the matrix effect of cassava materials, a preliminary recombination experiment was conducted by mixing the metabolite extracts of cassava organs with standard compound mixtures containing 1.0 μg of each metabolite and analyzed by GC-MS, with no significant matrix effect being observed in 41 out of 52 detected metabolites (Figure S9).

Determination of soluble sugars, starch and free amino acids

Soluble sugars, starch and free amino acids were extracted as described previously (Jung *et al.*, 2015). Levels of starch and soluble sugars and starch were determined using a NADP-coupled enzymatic test (Jung *et al.*, 2015) in 96-well plates and measured with a TECAN Infinite 200 Pro plate reader.

For determination of free amino acids, 20 μl from the ethanol extraction or 20 μl of a 1:10 diluted standard (Thermo Scientific Pierce Amino Acid Standard H) were mixed with 60 μl borate buffer (200 mM, pH 8.8) and 20 μl aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) solution (Synchem UG & Co. KG, Felsberg, Germany; in 2 mg ml^{-1} acetonitrile) vortexed immediately and heated at 55°C for 10 min. The derivatized AQC amino acids were quantified using a Dionex P680-HPLC system with an RF 2000 fluorescence detector (Dionex, Sunnyvale, CA, USA) and a column system consisting of CC8/4 ND 100-5 C18ec and CC 250/4 ND 100-5 C18ec (Macherey-Nagel, Düren, Germany). For separation of amino acids, a gradient consisting of 100 mM sodium acetate/7 mM triethanolamine (pH 5.2, buffer A) and acetonitrile/water (90%, buffer B) (0–100%) was used. The AQC amino acids were detected by fluorescence with an excitation wavelength at 254 nm and emission at 395 nm. Matrix effects on the measurement were minor for these metabolites (Figure S10).

Determination of enzyme activity

Enzyme activities were assayed as described in Gibon *et al.* (2004) and modified as in Table S3. The optimization of the enzymes measured was carried out with cassava plant material (TMEB419) grown under greenhouse conditions. The recovery of enzyme activities in each tissue after lyophilization was determined before analysis, as presented in Table S4.

Statistical analysis

All statistical analysis was carried out using R (v.3.4.3 or v.3.5.0). PCA was performed for the greenhouse and field data separately. The contents of each metabolite were normalized by the mean of these from all samples. The normalized metabolite contents were averaged for each tissue from each genotype. PCA was conducted using `prcomp` function with the default parameters without scaling; the score and loading plots were drawn by `autoplot` function in the `ggfortify` package. The top 20 metabolites that contributed in the separation into PC1 and PC2 were identified based on squared coordinates values calculated using the `get_pca_var` function in the `factoextra` package. A heatmap with hierarchical clustering was drawn using both greenhouse and field data together. The contents of each metabolite were normalized by the median

of these from all samples and the mean values for each tissue from each genotype in each growth environment were \log_2 transformed. A heatmap was drawn using the `heatmap` function in the `heatmap` package without clustering the samples. Variation of the contents of each metabolite among tissues was analyzed using both greenhouse and field data together. The raw metabolite contents data were analyzed using one-way analysis of variance (ANOVA) with the `aov` function using tissues in each growth environment as a factor. Tissues from individual plants were considered as replicates in both greenhouse ($n = 24$) and field ($n = 36$) experiments. Next, a Tukey honestly significant difference (HSD) test was performed using the `glht` function in the `multcomp` package and the results were presented as letters generated by `clt` function in the `multcomp` package. Boxplots were drawn using the `ggplot` function of the `ggplot2` package. Genotypic variation of agronomic and metabolic traits was analyzed for each tissue from greenhouse and field experiments separately using one-way ANOVA and cultivars as a factor. Pearson correlation analysis between metabolite contents and yield traits was performed by `cor.test` function and scatter plots were drawn by `ggplot` function. Metabolite contents and yield traits from a single plant were considered as a replicate for the greenhouse trial ($n = 24$, 4 plants \times 6 cultivars). For the correlation analysis between metabolite contents and yield parameters at 16 WAP in the field trial, a single plant was considered as a replicate ($n = 36$, 6 plants \times 6 cultivars). Means of metabolite contents from two plants in the same plot were analyzed against the yield parameters at 52 WAP derived from the same plot ($n = 18$, 3 plots \times 6 cultivars). A correlation *P*-value below 3.125×10^{-5} was considered as significant based on Bonferroni correction to the multiple analysis (1600 multiple analyses).

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AUTHOR CONTRIBUTIONS

AG, LAM, SCZ, MS, US, HEN, and ARF conceived and supervised the project. TO, LRS, MS, and ARF wrote the manuscript with the contributions of all authors. FL and WZ conducted the greenhouse experiment. AG and LS conducted the field experiments. TO, PAWK, LRS, and FL conducted biochemical analyses. AS measured enzyme activities. NM and LAM managed the data. TO, LRS, AS, MS, and ARF analyzed the data.

SUPPORTING INFORMATION

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

Data S1. Absolute contents of metabolites in the tissues of six cassava cultivars.

Data S2. Contents of all metabolites in each tissue of greenhouse and field-grown cassava plants.

Data S3. Correlation analysis of metabolite contents and yield parameters.

Data S4. Genotypic variation in contents of all metabolites in source leaf, sink leaf, stem, and storage root of cassava plants.

Data S5. Enzyme activities in the tissues of six cassava cultivars.

Figure S1. Contents of metabolites which contributed the most in the separation of tissues in the principal component analysis shown in the Figure 2 in each tissue of greenhouse and field-grown cassava plants.

Figure S2. Contents of the minor amino acids and their derivatives (which accumulated less than 1 mg g⁻¹ in any tissues) in each tissue of greenhouse and field-grown cassava plants.

Figure S3. Contents of the minor amino acids and their derivatives (which accumulated less than 1 mg g⁻¹ in any tissues) which showed tissue-specific accumulation only in the field-grown samples in each tissue of greenhouse and field-grown cassava plants.

Figure S4. Contents of the organic acids and sugar phosphates, which showed tissue-specific accumulation in each tissue of greenhouse-grown and field-grown cassava plants.

Figure S5. Genotypic variation of sucrose and starch contents in each tissue of cassava plants.

Figure S6. Genotypic variation of enzyme activities in the field in Nigeria in 2015 and 2016, and in the greenhouse in Erlangen, Germany.

Figure S7. Recovery rates of organic acids and phosphorylated intermediates in cassava storage root, stem, fibrous root and source leaf tissue.

Figure S8. Effects of freeze drying in the detection of individual metabolites in all cassava organs for metabolic profiling.

Figure S9. Evaluation of matrix effects on the detection of individual metabolites in all cassava organs.

Figure S10. Evaluation of matrix effects on the detection of individual metabolites in all cassava organs.

Table S1. List of cassava genotypes used in this study and their characteristics.

Table S2. List of enzymes, abbreviations and their assignment to pathways.

Table S3. Optimization of enzyme activity assays.

Table S4. Recovery of enzyme activities after lyophilization.

Table S5. Comparison of RuBisCO and PEP carboxylase activities in mature maize leaves, Arabidopsis standard material and greenhouse-grown cassava.

Table S6. Comparison of the starch synthesis enzyme AGPase and the glycolytic enzyme, PFK and PK activities in storage roots and stems of field-grown and greenhouse-grown cassava cultivars.

Table S7. Results of composition analysis for substrate used in the greenhouse, Germany and soil in experimental plots in the field in Nigeria.

Table S8. Overview of experimental details, conditions and analysis. Abbreviation: GH, greenhouse.

OPEN RESEARCH BADGES



This article has earned an Open Data Badge for making publicly available the digitally shareable data necessary to reproduce the reported results. The data is available at ftp://cassavabase.org/CASS/CASS_6Genotypes_Metabolites.csv.

This article has earned an Open Materials Badge for making publicly available the components of the research methodology needed to reproduce the reported procedure and analysis.

DATA AVAILABILITY STATEMENT

All relevant data can be found within the manuscript and its supporting information.

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