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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 the greenhouse in Erlangen, Germany and 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:phospho*enol*pyruvate carboxylase activity support a C_3 mode of photosynthesis. The high rate of photosynthesis is likely 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 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 enhance sink capacity in the high light and low nitrogen environment in the field. Overall, the results indicate that carbon assimilation rate, the K battery, root starch synthesis, trehalose, and chlorogenic acid accumulation are potential target traits for genetic improvement.

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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 (FAO, 2016). In many countries of sub-Saharan Africa cassava leaves also provide an important source of protein, vitamins and micronutrients for humans and livestock . 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 reveal that cassava has overtaken soybean and potato in terms of calorific yield (FAO, 2016).

Despite extensive research on disease susceptibility and post-harvest deterioration, relatively little 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 whilst growing on impoverished soils remain unanswered or, in the case of the first question, were only recently answered (Arrivault *et al.*, 2019). Earlier publications varyingly claimed that cassava is either a C_3 (Calatayud *et al.*, 2002), a C_4 or a C_3-C_4 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 C_4 enzyme PEP carboxylase (El-Sharkawy, 2004). However, cassava does not display Kranz anatomy (El-Sharkawy, 2004) and archeological isotope dating on conserved stomach contents give consistent ages for cassava to those for plants that are well established to employ the C_3 mode of photosynthesis. To directly address this question, $^{13}CO_2$ feeding experiments were performed on cassava and the metabolic fate of the ^{13}C followed by mass-spectrometry and shown to be very similar to the C_3 plant Arabidopsis (Szecowka *et al.*, 2013) and different to the C_4 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

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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 to the high yields in South Asia where cassava is fertilized (FAO, 2018). On the other hand, over-fertilization causes phenological changes that enhance shoot growth at the cost of root production (Pellet and El-Sharkawy, 1993). 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 sink since 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.*,

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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 inter-cultivar 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 inter-cultivar 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 determined also after one 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

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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 in 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 (Figure 2b & d, Figure 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 contains putrescine, glutamine, ornithine, arginine, asparagine, serine, proline, nitrate, and trehalose. However, tissue specific accumulation of these metabolites was less evident in field- 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 mg g^{-1} and 5 mg g^{-1} in the stem of

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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. Contrary, 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).

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- and field-grown plants except that the sink leaf content was slightly higher in greenhouse-grown plants (Figure 4). Source leaf- and stem-specific accumulation of trehalose was found in greenhouse- 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 reveals that source and sink leaves accumulated more starch in field than in 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 3-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- 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

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are shown in Data S3, and genotypic variation of metabolite levels in each tissue is shown for all metabolites in Data S4.

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 acid, 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, and trehalose in both the sink and source leaves showed a positive correlation with harvest index at 16 WAP (Figure 5b). These positive correlations depend 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 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 (Table S3, S4). In all analysis

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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 (Fig 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 grouped according to trial, rather than 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 (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). On the other hand, many enzymes that are involved exclusively in respiratory metabolism (G6PDH, pyruvate kinase, fructokinase, glucokinase, isocitrate 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 to 8.1).

AGPase activity in roots was consistently about 2-fold higher in field- than greenhouse-grown material (Table S6, Figure S6b). This contrasts with the glycolytic enzymes PFK and 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- compared to greenhouse-grown plants (see above). AGPase, PFK, and PK activities were all higher in the

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stems of field- 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 (FAO 2016). 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 considerable and 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 to 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 is most likely due to the increase of 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 indicates 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 positively with trehalose, 3-trans-

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caffeoylquinic acid (chlorogenic acid) and AMP (Figure 5). Some of these relationships point to a relation 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) signaling 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 signaling 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 leads to substrate leakage (Minchin and Thorpe, 1987), which high concentrations of K in the phloem serve to counteract.

Cassava yield is mainly source- and sink-limited in 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

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availability in the greenhouse than in the field. This is likely to lead to stronger source limitation in the greenhouse- compared to 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 CO_2 (Rosenthal *et al.*, 2012).

De Souza & Long (2019) 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 2- to 3-fold increase in AGPase activity (Figure S6b) and a 3-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 indicate 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 coordinated increase in the activity of other enzymes or transporters involved in sucrose breakdown but, notably, it was not accompanied by an increase in 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-signaling and possibly negative effects of nitrogen signaling (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 signaling 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 point 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 to 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

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highlights the importance of the difference in AGPase activity that we observed between greenhouse and field conditions.

Taken together, cassava root yield is likely 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 will likely require intervention in both source and sink function. At first sight, this conclusion is unexpected, as cassava has very high rates of photosynthesis for a C_3 species (see above). However, photosynthesis rates including cumulative photosynthesis over a season are not always high, and photosynthetic efficiency is only ~14% of the theoretical maximum for a C_3 plant (De Souza *et al.*, 2017). Further, 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 (Figure 3, 4, and S1-S5). In both greenhouse- and field-grown plants, the storage root was characterized by high starch and low protein, whilst the stem was characterized by high levels of glucose, fructose and glutamine and high linamarin (Figure 2 and 4). A different picture appears for nitrogen-containing primary metabolites, with most of these being considerably higher in greenhouse- than field-grown plants (Figure 4). This is almost certainly due to the paucity of soil nitrogen in Ibadan with total nitrogen representing 0.07 % of soil mass in Ibadan, compared to 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 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 which 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 suggest 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,

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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 impoverished 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- than greenhouse-grown material, pointing to the ability of cassava to maintain photosynthesis capacity in low nitrogen conditions. Second, increased tuber yield in the field compared to the greenhouse was associated with lower amino acid levels in many organs (Figure 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- and field-grown plants (Figure 4). These amino acids likely serve for nitrogen storage and 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 field-material (Figure 4) suggesting that linamarin likely serve as an N storage compound. Linamarin is considered a nitrogen storage molecule (Siritunga and Sayre, 2004) since 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 condition 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 indicate 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) reveals similar trends and similar ranges of absolute levels of most

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nitrogen-rich metabolites across all these species (Data S1). However, asparagine and glutamine were considerably higher in cassava than tomato leaves whilst 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 indicate 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 since 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 to 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 C_3 mode of photosynthesis, our results reveal that the high rate of photosynthesis in cassava is likely due to high activities of photosynthetic enzymes. Second, storage root formation in cassava is likely co-limited by both sink and source capacity, with the interaction to the growth conditions. Third, yield is remarkably 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 to temperate crops. Glutamine and putrescine accumulated in the stem under the nitrogen rich environment most probably serving as forms of nitrogen storage, which may partially support the high nitrogen use efficiency of cassava plants. More generally,

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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 highlights photosynthetic carbon assimilation rate (source capacity) under photosynthetically unfavorable condition, the K battery, the 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 – October, 2015) and in one experimental field in Ibadan, Nigeria (May, 2015 – August 2016). The genotypes were chosen to cover diversity in yield, flowering time, resistance to cassava mosaic virus (CMV) 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-liter pot and grown in a 20 m^2 chamber from August 4th, 2015 until October 29th, 2015. Growth light was supplemented with 250 µmol m^{-2} s^{-1} in 12/12 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 the same genotype clustered together. The positions of these plants were rotated twice a week. In the 2015-2016 field trial, plants were planted May 28th, 2015. The sampling took place in the week of the September 21st, 2015, when it is still in the high period of the first rainy season to avoid metabolic and physiological changes by drought. The layout was a Randomized Complete Block Design where three blocks (replicates) containing each 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 - July 2017. The material from 2016-2017 field trial was used only for the enzyme activity measurements. The experimental design was the same as that of 2015-2016 trial except that two genotypes, IITA-

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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 are 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 weeks after planting (WAP), two individual plants per plot or pool of all plants (18 to 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 1m x 1m 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 prior to shipments. The samples were well ground to a fine powder to be well-mixed by motor and pestle and the 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 of freeze-dried cassava tissue according to (Horst *et al.*, 2010a; Horst *et al.*, 2010b). The recovery rate for each metabolite and tissue was determined prior to analysis by adding known quantities of authentic standards at the start of the metabolite determination process i.e. the 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 of freeze-dried and fine milled (Retsch) cassava tissue material, mixed thoroughly and heated for 15 min at 95 °C. After centrifugation (10 min, 16000 *g*), the supernatant was used for quantification via ion

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chromatography by a 761-IC compact device (Metrohm). The column system consisting of Metrosep A Supp 4/5 Guard 4.0 and Metrosep A Supp 4-250/4.0 (Metrohm), followed by conductometry at a flow rate of 1ml/min and 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 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 in a mixture consisting of 5 ml HNO_3 (65 %, v/v), 2 ml H_2O_2 (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 (Lisec *et al.*, 2006) using 10 mg of ground freeze-dried cassava tissue. Ribitol (0.2 mg/ml in H_2O) was used as internal standard during extraction. A dry aliquot of the polar phase was resuspended in methoxyaminhydrochlorid (20 mg/mL in pyridine) and derivatized using N-methyl-N-[trimethylsilyl] trifluoroacetamide (MSTFA) containing 20 µL/ml fatty acid methyl esters mixture (FAMEs) 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 amount of metabolites from the peak intensity of a representative fragment.

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The effects of freeze-drying in the detection of individual metabolites in each tissue was determined prior to analysis. 54 of 59 detected metabolites showed smaller 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 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 the 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 AQC solution (6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, Synchem UG & Co. KG, 2 mg/ml 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) and the column system consisting of CC8/4 ND 100-5 C18ec and CC 250/4 ND 100-5 C18ec (Macherey-Nagel). For the 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 to 100 %) was used. The AQC amino acids were detected by fluorescence with 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 activitieswere assayed as described in (Gibon *et al.*, 2004) and modified as in the 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 lyophilisation was determined prior to analysis as presented in Table S4.

Statistical analysis

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All statistical analysis was carried out by R (version 3.4.3 or 3.5.0). PCA was performed for the greenhouse and field data separately. The contents of each metabolite were normalized by the mean of them from all samples. The normalized metabolite contents were averaged for each tissue from each genotype. The PCA was conducted by prcomp function with the default parameters without scaling and the score and loading plots were drawn by autoplot function in ggfortify package. The top 20 metabolites which contributed in the separation in PC1 and PC2 were identified based on squared coordinates values calculated by get_pca_var function in factoextra package. Heatmap with hierarchical clustering was drawn using both greenhouse and field data together. The contents of each metabolite were normalized by the median of them from all samples and the mean values for each tissue from each genotype in each growth environment were $\log_2$ transformed. The heatmap was drawn by pheatmap function in pheatmap package without clustering the samples. Variation of the contents of each metabolite among tissues were analyzed using both greenhouse and field data together. The raw metabolite contents data was analyzed by one-way analysis of variance (ANOVA) with 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. Following Tukey honestly significant difference (HSD) test was performed by glht function in multcomp package and the results were presented as letters generated by cld function in multcomp package. Boxplots were drawn by ggplot function of ggplot2 package. Genotypic variation of agronomic and metabolic traits were analyzed for each tissue from greenhouse and field experiments separately by one-way ANOVA using 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 x 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 x 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 x 6 cultivars). The correlation p-value below 3.125×10^{-5} was considered as significant based on Bonferroni correction to the multiple analysis (1600 multiple analyses).

Data statement

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

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The authors declare no competing interests in this work

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.

Short Legends of Supporting Information

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.

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Figure S2. Contents of the minor amino acids and their derivatives (which accumulated lower 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 lower 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 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.

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. Optimisation of enzyme activity assays.

Table S4. Recovery of enzyme activities after lyophilisation.

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.

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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: Greenhouse, GH.

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Figure Legends

Figure 1. Agronomic traits of six commercial cassava cultivars in the greenhouse-grown plants at the 12 weeks after planting (A) and field grown plants at the 16 (B) and 52 week (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

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variation in yield traits among cultivars and the letters indicate the results of following Tukey HSD test comparing values among cultivars in the same condition ($p<0.05$).

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) and field (C) experiments and respective loading plots including the names of 20 variables contributed most in the separation in PC1 and PC2 (B and 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.

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 $\log_2$ transformed. The means of the values in each tissue from all cultivars were shown as a heatmap. Red and blue colors represent high and low levels of metabolites using false-color scale, respectively. Metabolites are arranged based on the result of hierarchical clustering and the Dendrogram is shown on the left.

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 HSD test comparing values among tissues in the same condition ($p<0.05$).

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 x 6 cultivars in the greenhouse, n=36; 6 plants x 6 cultivars in the field). Colors of the points correspond to cultivars. r and p are the correlation coefficient and P value from Pearson correlation analysis, respectively.

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

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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 HSD test comparing values among cultivars ($p<0.05$).

Figure 7. Enzyme activities in mature cassava leaves from the cultivar TME419/TMEB419 compared to 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 CBC, starch and sucrose synthesis and PEPC were also seen in the other cultures and, for enzymes that could be measured, in the field-grown plants (Data S5, Figure S6).

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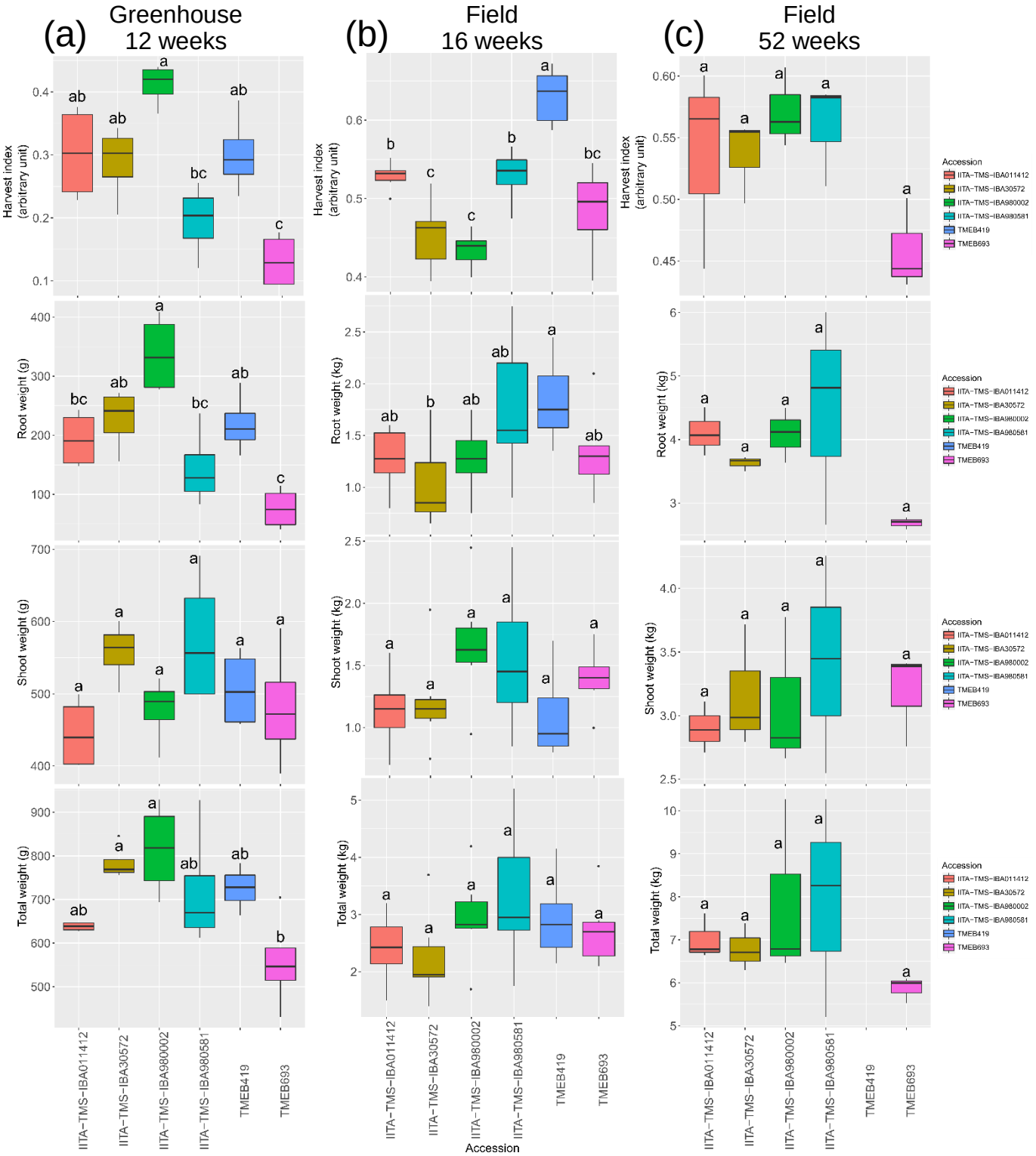
(a)
Greenhouse
12 weeks
(b)
Field
16 weeks
(c)
Field
52 weeks
Harvest index (arbitrary unit)
Root weight (g)
Root weight (kg)
Shoot weight (g)
Shoot weight (kg)
Total weight (g)
Total weight (kg)
Accession
IITA-TMS-IBA011412
IITA-TMS-IBA30572
IITA-TMS-IBA980002
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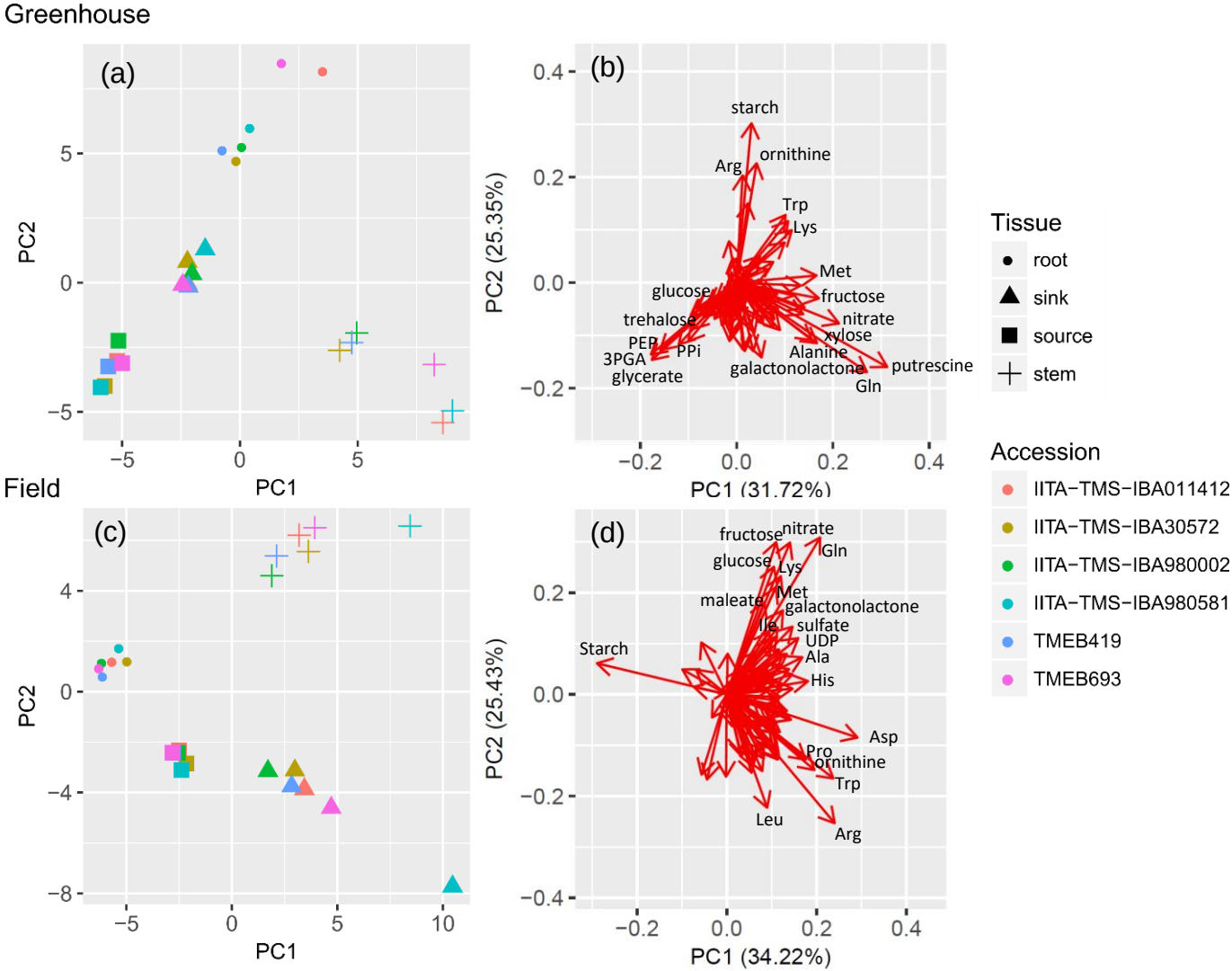
TMEB419
TMEB693


Greenhouse
(a)
PC2
PC1
5
0
−5
−5
0
5
(b)
PC2 (25.35%)
PC1 (31.72%)
0.4
0.2
0.0
−0.2
−0.2
0.0
0.2
0.4
starch
ornithine
Arg
Trp
Lys
Met
fructose
nitrate
xylose
Alanine
putrescine
Gln
galactonolactone
glucose
trehalose
PEP
3PGA
glycerate
PPi
Tissue
root
sink
source
stem
Field
(c)
PC2
PC1
4
0
−4
−8
−5
0
5
10
(d)
PC2 (25.43%)
PC1 (34.22%)
0.2
0.0
−0.2
−0.4
−0.2
0.0
0.2
0.4
fructose
nitrate
Gln
glucose
Lys
Met
maleate
galactonolactone
Ile
sulfate
UDP
Starch
Ala
His
Asp
Pro
ornithine
Trp
Leu
Arg
Accession
IITA−TMS−IBA011412
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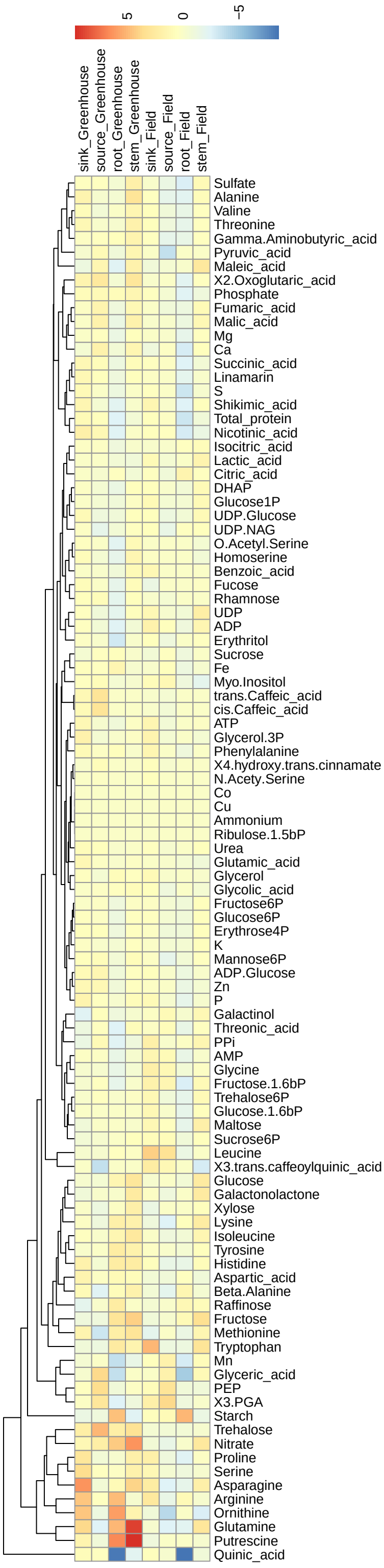
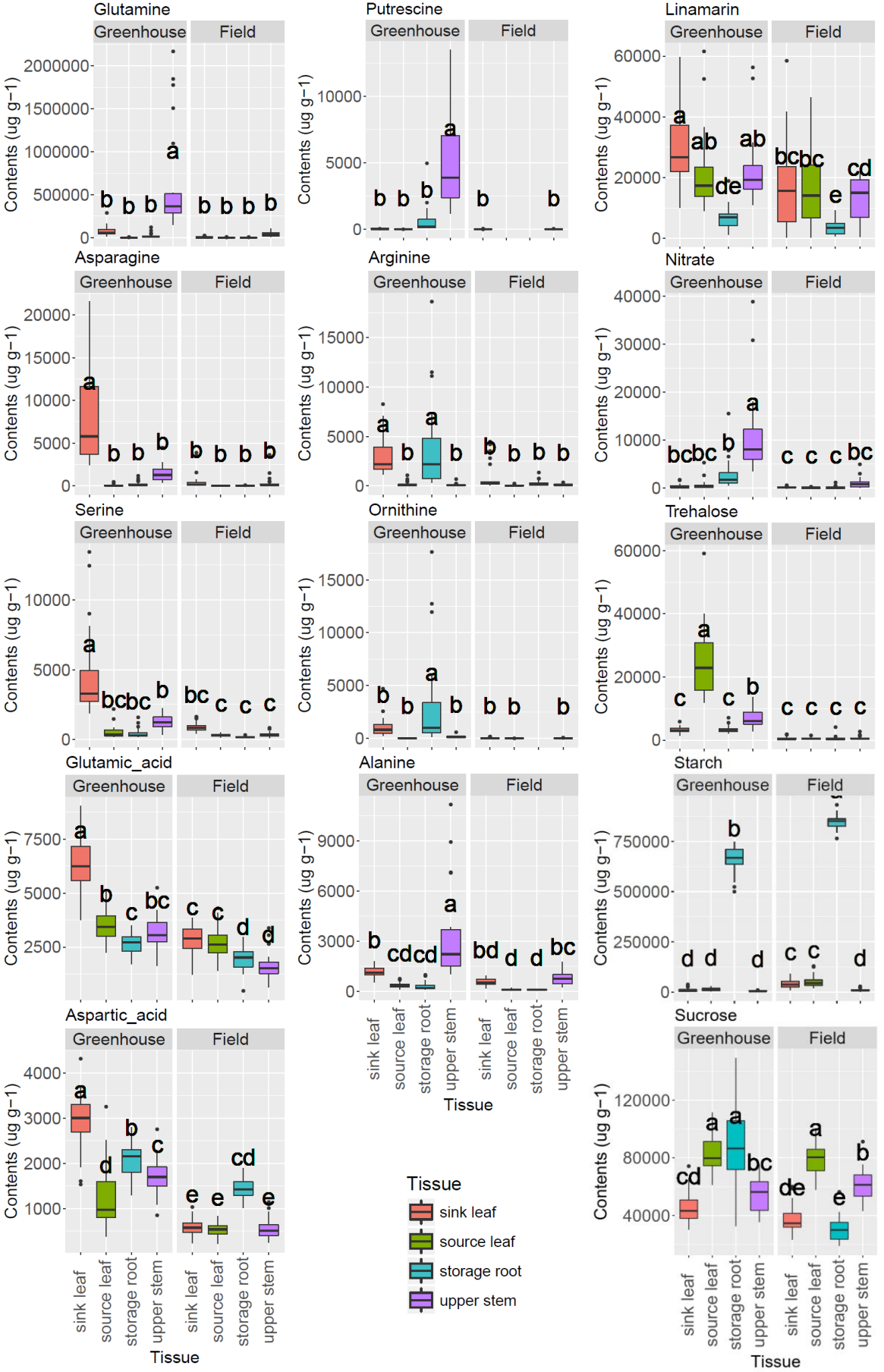
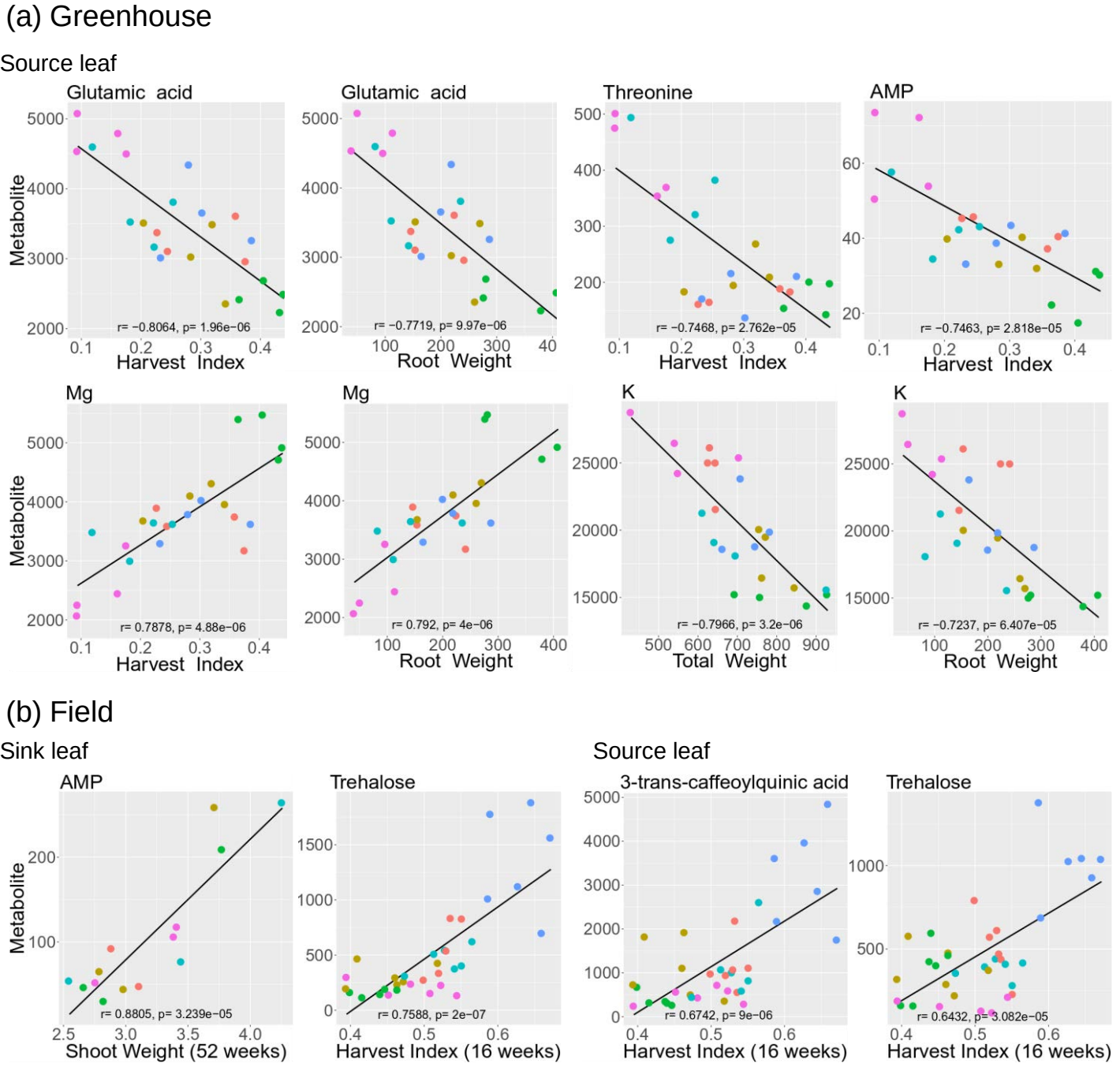
IITA−TMS−IBA980581
TMEB419
TMEB693

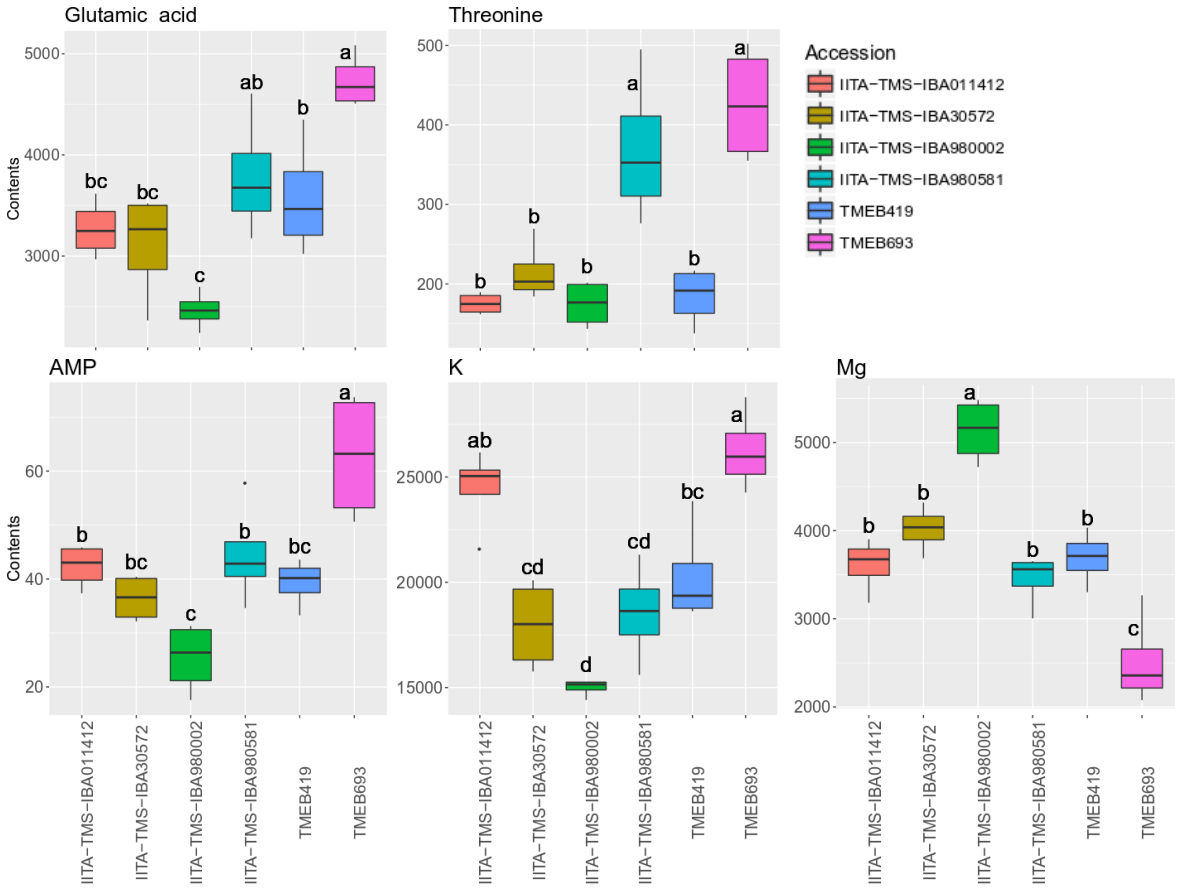

5
0
−5
sink_Greenhouse
source_Greenhouse
root_Greenhouse
stem_Greenhouse
sink_Field
source_Field
root_Field
stem_Field
Sulfate
Alanine
Valine
Threonine
Gamma.Aminobutyric_acid
Pyruvic_acid
Maleic_acid
X2.Oxoglutaric_acid
Phosphate
Fumaric_acid
Malic_acid
Mg
Ca
Succinic_acid
Linamarin
S
Shikimic_acid
Total_protein
Nicotinic_acid
Isocitric_acid
Lactic_acid
Citric_acid
DHAP
Glucose1P
UDP.Glucose
UDP.NAG
O.Acetyl.Serine
Homoserine
Benzoic_acid
Fucose
Rhamnose
UDP
ADP
Erythritol
Sucrose
Fe
Myo.Inositol
trans.Caffeic_acid
cis.Caffeic_acid
ATP
Glycerol.3P
Phenylalanine
X4.hydroxy.trans.cinnamate
N.Acety.Serine
Co
Cu
Ammonium
Ribulose.1.5bP
Urea
Glutamic_acid
Glycerol
Glycolic_acid
Fructose6P
Glucose6P
Erythrose4P
K
Mannose6P
ADP.Glucose
Zn
P
Galactinol
Threonic_acid
PPi
AMP
Glycine
Fructose.1.6bP
Trehalose6P
Glucose.1.6bP
Maltose
Sucrose6P
Leucine
X3.trans.caffeoylquinic_acid
Glucose
Galactonolactone
Xylose
Lysine
Isoleucine
Tyrosine
Histidine
Aspartic_acid
Beta.Alanine
Raffinose
Fructose
Methionine
Tryptophan
Mn
Glyceric_acid
PEP
X3.PGA
Starch
Trehalose
Nitrate
Proline
Serine
Asparagine
Arginine
Ornithine
Glutamine
Putrescine
Quinic_acid


Glutamine
Putrescine
Linamarin
Asparagine
Arginine
Nitrate
Serine
Ornithine
Trehalose
Glutamic_acid
Alanine
Starch
Aspartic_acid
Sucrose
Greenhouse
Field
Contents (ug g−1)
Tissue
sink leaf
source leaf
storage root
upper stem


(a) Greenhouse
Source leaf
Glutamic acid
Metabolite
r= −0.8064, p= 1.96e−06
Harvest Index
Glutamic acid
r= −0.7719, p= 9.97e−06
Root Weight
Threonine
r= −0.7468, p= 2.762e−05
Harvest Index
AMP
r= −0.7463, p= 2.818e−05
Harvest Index
Mg
Metabolite
r= 0.7878, p= 4.88e−06
Harvest Index
Mg
r= 0.792, p= 4e−06
Root Weight
K
r= −0.7966, p= 3.2e−06
Total Weight
K
r= −0.7237, p= 6.407e−05
Root Weight
(b) Field
Sink leaf
AMP
Metabolite
r= 0.8805, p= 3.239e−05
Shoot Weight (52 weeks)
Trehalose
r= 0.7588, p= 2e−07
Harvest Index (16 weeks)
Source leaf
3-trans-caffeoylquinic acid
r= 0.6742, p= 9e−06
Harvest Index (16 weeks)
Trehalose
r= 0.6432, p= 3.082e−05
Harvest Index (16 weeks)

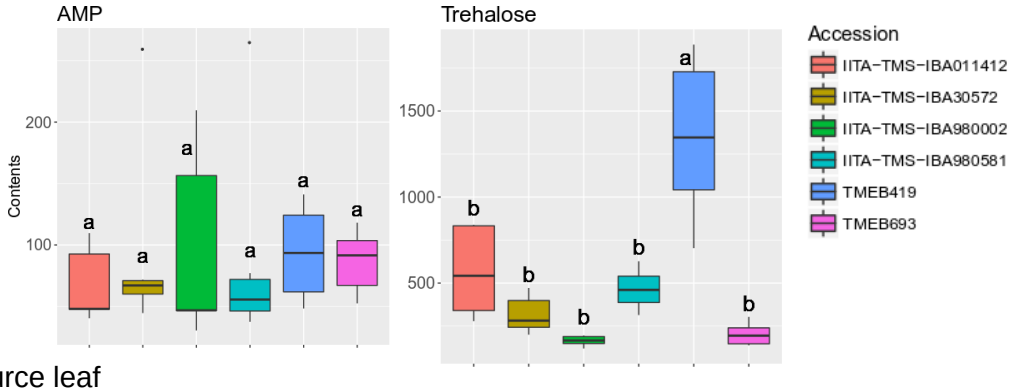
(a) Greenhouse

Source leaf

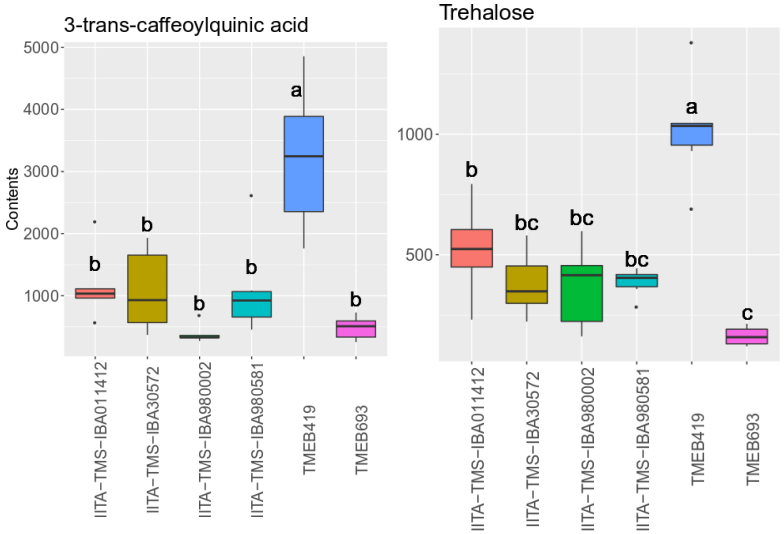


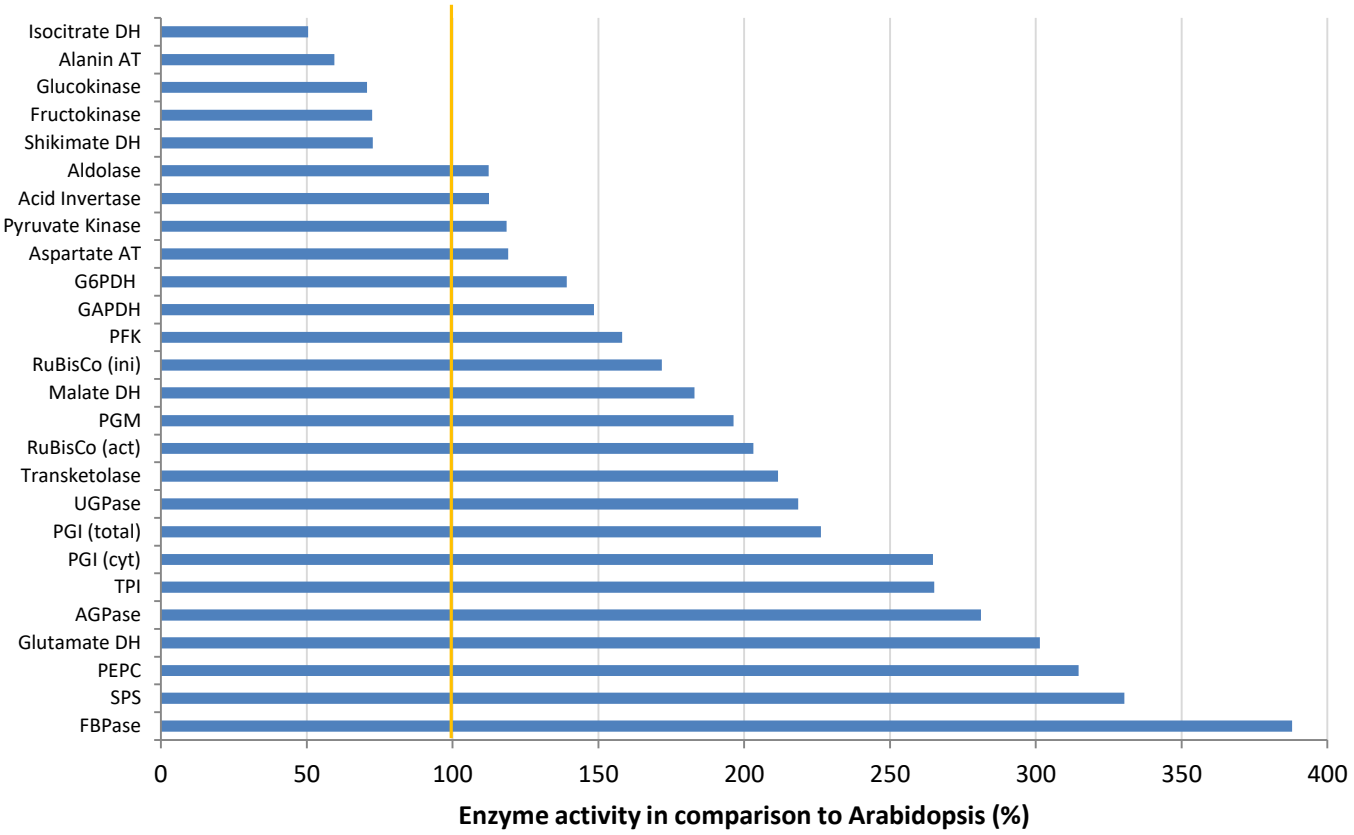
(b) Field

Sink leaf



Source leaf




Isocitrate DH
Alanin AT
Glucokinase
Fructokinase
Shikimate DH
Aldolase
Acid Invertase
Pyruvate Kinase
Aspartate AT
G6PDH
GAPDH
PFK
RuBisCo (ini)
Malate DH
PGM
RuBisCo (act)
Transketolase
UGPase
PGI (total)
PGI (cyt)
TPI
AGPase
Glutamate DH
PEPC
SPS
FBPase
0
50
100
150
200
250
300
350
400
Enzyme activity in comparison to Arabidopsis (%)