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Research

# Root metabolome of plant–arbuscular mycorrhizal symbiosis mirrors the mutualistic or parasitic mycorrhizal phenotype

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## Summary

- The symbiosis of arbuscular mycorrhizal fungi (AMF) with plants, the most ancient and widespread association, exhibits phenotypes that range from mutualism to parasitism. However, we still lack an understanding of the cellular-level mechanisms that differentiate and regulate these phenotypes.
- We assessed the modulation in growth parameters and root metabolome of two sorghum accessions inoculated with two AMF species (*Rhizophagus irregularis*, *Gigaspora gigantea*), alone and in a mixture under phosphorus (P) limiting conditions. *Rhizophagus irregularis* exhibited a mutualistic phenotype with increased P uptake and plant growth.
- This positive outcome was associated with a facilitatory metabolic response including higher abundance of organic acids and specialized metabolites critical to maintaining a functional symbiosis. However, *G. gigantea* exhibited a parasitic phenotype that led to plant growth depression and resulted in inhibitory plant metabolic responses including the higher abundance of *p*-hydroxyphenylacetaldoxime with antifungal properties.
- These findings suggest that the differential outcome of plant–AMF symbiosis could be regulated by or reflected in changes in the root metabolome that arises from the interaction of the plant species with the specific AMF species. A mutualistic symbiotic association prevailed when the host plants were exposed to a mixture of AMF. Our results provide a metabolome-level landscape of plant–AMF symbiosis and highlight the importance of the identity of both AMF and crop genotypes in facilitating a mutualistic AMF symbiosis.

## Introduction

The association of plant roots with arbuscular mycorrhizal fungi (AMF) is the most ancient and widespread symbiosis in terrestrial ecosystems. Under low soil nutrient availability, AMF, through its extensive hyphal networks, provides 70–80% of the plants' phosphorus (P) requirement (Smith & Read, 1997). The AMF symbiosis also increases plant tolerance to drought, salt stress, pests, and diseases (Pozo & Azcón-Aguilar, 2007; Ruiz-Lozano *et al.*, 2011), enhances the quality of agricultural products (Baum *et al.*, 2015), and facilitates the accrual of soil carbon (Wilson *et al.*, 2009). In return for resource foraging, plants provide carbon to AMF, accounting for *c.* 4–20% of the plant photosynthates (Johnson *et al.*, 1997). Due to their capacity to capture residual soil nutrients that are less bioavailable, there is a high potential to utilize AMF symbiosis in sustainable agriculture (Rillig *et al.*, 2019). However, the high functional diversity of AMF often results in contrasting outcomes in terms of plant productivity across different plant–AMF combinations (Feddermann *et al.*, 2010; Mensah *et al.*, 2015). The underlying mechanisms for this differential outcome are less known, which precludes the large-scale utilization of AMF symbiosis in agriculture.

In plant–AMF symbiosis, based on the functional pairing of AMF and the host plant, the net benefit in plant productivity can shift from being positive to negative (Johnson *et al.*, 1997; Graham & Abbott, 2000; Klironomos, 2000; Taylor & Harrier, 2000; Hart & Reader, 2002; Munkvold *et al.*, 2004; Mensah *et al.*, 2015). The breakdown of mutualism between plants and beneficial microbes could result from the conflict of interests arising from the net cost involved for both partners to maintain a symbiotic association (Sachs & Simms, 2006). Since the same plant can associate with multiple AMF species, mutualism can be achieved by stabilizing mechanisms such as partner choice or plant sanctions (Kiers & Denison, 2008). Plants can preferentially allocate resources to the most cooperative (in terms of resource transaction) AMF partners and thus eliminate the less cooperative partners (Kiers & Van Der Heijden, 2006). However, plant–AMF symbiosis could also result in a parasitic phenotype where plant growth is reduced due to symbiosis (Johnson *et al.*, 1997). Thus, despite stabilizing mechanisms, plant and AMF genotype combinations can lead to noncooperative behaviors in specific environmental conditions. It is less known whether plants attempt to inhibit the AMF associations that result in a parasitic phenotype and the mechanisms of such regulations.

The benefit conferred by AMF to plants is context-dependent and varies with the plant species/genotypes, the AMF genera/species/isolates (Klironomos, 2003; Sanders & Rodriguez, 2016; Koch *et al.*, 2017), and the environmental conditions (Johnson *et al.*, 1997). Although the same plant species can be colonized by diverse AMF genera/species/isolates (low specificity of association), the overall productivity of the plant is dependent on the identity of the associated AMF (high specificity of the outcome). AMF belonging to the taxon Gigasporaceae (genus *Gigaspora*) occasionally exhibits a parasitic phenotype compared to the members of Glomeraceae (genus *Rhizophagus*). In *Medicago truncatula*, wheat, and ryegrass, the inoculation of *Gigaspora margarita* resulted in growth depression (Buwalda & Goh, 1982; Li *et al.*, 2008; Lendenmann *et al.*, 2011). Similarly, across different sorghum accessions, *Gigaspora gigantea* caused a negative mycorrhizal growth response (MGR) compared to other AMF species belonging to *Rhizophagus* and *Clarideoglomus* genera (Watts-Williams *et al.*, 2019). A recent meta-analysis also concluded that members of the Glomeraceae had the maximum P uptake efficiency and pathogen inhibition capacity compared to members of Gigasporaceae (Yang *et al.*, 2015). This specificity of the outcome in plant–AMF symbiosis is also evident in studies that used mixtures of multiple AMF species (Wagg *et al.*, 2011). However, the molecular-level mechanisms underlying this difference in the outcome of the plant–AMF symbiosis are not known.

The morphological and physiological traits of the members of Gigasporaceae and Glomeraceae vary widely. The members of Gigasporaceae may be classified as competitive AMF as they are characterized by the higher production of extraradical hyphae that can explore large volumes of soil to obtain P (Chagnon *et al.*, 2013) and might demand greater carbon from the host plant (Lendenmann *et al.*, 2011). In contrast, the members of the Glomeraceae are more stress-tolerant since they form hyphae closer to the roots that help them withstand disturbances such as tillage (Parniske, 2008; Chagnon *et al.*, 2013). These differences in the morphological and physiological traits of the AMF species can partly contribute to the level of functional compatibility between the plant–AMF combinations and hence their outcome (Parniske, 2008). The AMF species belonging to the genera *Rhizophagus* and *Gigaspora* also differ in their effector proteins, which suppress the defense responses of the host plant. The difference in effector proteins could potentially lead to variations between these genera in their host range (Kamel *et al.*, 2017). Recent research has revealed that apart from fungal effectors, other molecular features such as signaling molecules are more critical in driving plant growth benefits than the AMF growth and morphology (Lo Presti *et al.*, 2015; Kamel *et al.*, 2017; Koch *et al.*, 2017; Lanfranco *et al.*, 2018). Many of the molecules that have a critical role in plant–AMF communication and the establishment of a functional symbiosis are the plant secondary (Kaur & Suseela, 2020) or the specialized metabolites (Alseekh & Fernie, 2018; Weng *et al.*, 2021). Thus, besides the differences in the functional traits and molecular features between the different AMF species, the metabolic phenotype of the host plant is critical in shaping the outcome of the plant–AMF symbiosis. The plant metabolic phenotype results from the inherent metabolic composition of the plant and their modulation through the interaction of the plant species with the specific AMF species (Schweiger *et al.*, 2014; Rivero *et al.*, 2015, 2018). There are no comparisons to date into the plant metabolic response with AMF isolates expressing mutualistic or parasitic phenotypes.

Understanding the dynamics of the phytometabolome under a mutualistic or parasitic AMF phenotype can provide a better molecular insight into the differential outcome of the plant–AMF symbioses. Specialized metabolites play a significant role in all stages of plant–AMF symbiosis, from spore germination and hyphal branching to establishing a functional symbiosis (Akiyama *et al.*, 2005; Genre *et al.*, 2013). In plant–AMF symbioses, the modulation of plant specialized metabolome could avoid the antagonistic defense responses of the plant and prime the plants for better tolerance to environmental stresses. As with specialized metabolites, the composition and exchange of primary metabolites could also influence the outcome of this symbiotic association, especially since the AMF depends solely on photosynthates as their source of energy. For example, an efficient transfer of primary metabolites could facilitate a mutualistic phenotype by providing more carbon to the fungi. Different AMF species could differentially modulate the plant metabolome to establish a functional symbiosis. Hence, the specificity in the outcome of plant–AMF symbiosis could be regulated by or reflected in changes in the primary and specialized plant metabolome (Kaur & Suseela, 2020).

Previous metabolomic studies on plant–AMF symbiosis were mostly targeted analyses focusing on specific metabolites (Stumpe *et al.*, 2005; Berruti *et al.*, 2016), while a few recent studies with untargeted metabolomic analyses concentrated on the modulations of phytometabolome resulting from AMF symbiosis that exhibits a mutualistic phenotype (Schweiger *et al.*, 2014; Rivero *et al.*, 2015, 2018). However, to our knowledge, none of the previous studies have investigated the differential metabolic reprogramming between a mutualistic and a parasitic plant–AMF association, which is critical in elucidating the processes regulating the specificity in the outcome of this symbiosis. The three main questions that we addressed in this study are (1) Does the mutualistic or parasitic phenotype of plant–AMF symbiosis produce a unique phytometabolome? (2) Are there facilitatory or inhibitory metabolic responses of the plant when the AMF symbiosis exhibits a mutualistic or parasitic phenotype, respectively? (3) Does this pattern of phytometabolome vary with different plant genotypes or vary strictly with the fungal identity? We hypothesized that the specificity of the plant–AMF association (the mutualistic or parasitic phenotype) arises partly from the ability of the AMF species to differentially modulate the primary and specialized metabolites of the plants to their advantage. Different AMF genera were shown to elicit contrasting growth responses (mutualistic vs parasitic) within a single plant genotype (Watts-Williams *et al.*, 2019). The AMF identity, which partly regulates the direction of the symbiotic outcome, may have an overriding effect on the plant chemotype. Thus, we also hypothesized that the metabolic reprogramming in roots, irrespective of the genotypic identity of the plant, will be highly AMF dependent. We used sorghum (*Sorghum* spp.) as a model species to test

these hypotheses. Here we used two different sorghum accessions (PI-297130 and PI-562730) that exhibited opposite trends regarding MGR when inoculated with different AMF species (Watts-Williams *et al.*, 2019). The accession PI-297130 that belongs to *Sorghum caudatum* showed a negative MGR when inoculated with *G. gigantea* while, PI-562730 which belongs to *Sorghum bicolor* exhibited a positive MGR when inoculated with four different AMF species, including *G. gigantea* and *Rhizophagus irregularis* (Watts-Williams *et al.*, 2019).

## Materials and Methods

### Experimental setup

Seeds of the *S. caudatum* accession PI-297130 and *S. bicolor* accession PI-562730 (kindly provided by Dr Stephen Kresovich) were surface sterilized with 70% ethanol, rinsed with distilled water, and planted to 6-l pots with autoclaved river sand (particle size: 0.06−2 mm). The AMF treatments included a single species inoculum of *R. irregularis* isolate FL208A (hereafter Rhizophagus treatment) or *G. gigantea* isolate MN922A (Gigaspora treatment), a 1 : 1 (v/v) mixture of both the above AMF species (MIX treatment) and a noninoculated control treatment (NAM). We optimized the volume of the inoculum (10 ml) in each AMF treatment pot based on preliminary experiments. The sand-based AMF inoculum obtained from the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM, West Virginia University, Morgantown, WV, USA) had both spores and mycelium and were multiplied on Sudan grass (*Sorghum* × *drummondii*). Each treatment was replicated six times. We filled each pot with 7.1 kg of sand supplied with 7 g of sparingly soluble iron phosphate ($FePO_4$; Ksp (solubility product constant) = $1.3 \times 10^{-22}$) to create P-limiting conditions, which is equivalent to 1.44 g P per pot. After filling the pot with 3 l of sand, the AMF inoculum was mixed with another 1 l of sand and added as a band of high spore density to ensure maximum colonization. The top-most layer of 1 l of sand was added without any inoculum and the pots were arranged in a randomized block design. Plants were supplied with 400 ml of full-strength Hoagland solution without P every 4 d and watered with deionized water as needed (details in Supporting Information Methods S1). The experiment was conducted in 2019 in the glasshouse at Clemson University, Clemson, South Carolina, in June and July with a day : night temperature of 27°C : 23°C, 16 h per day length, and under supplemental lighting conditions (350–400 W $m^{-2}$).

### Plant harvest and sample analyses

After 30 d of growth, the plants were destructively harvested and separated into shoots and roots and the fresh weight was recorded. The shoots were oven-dried at 60°C for 48 h, and the shoot dry weight (SDW) was recorded. The roots were gently washed with distilled water to remove the adhering sand. The finer lateral roots were separated from the remaining roots and used for various analyses as described later. For metabolomics analysis, a subsample of the fine roots was immediately frozen and stored at −80°C. Although the metabolic responses to AMF may differ between the aboveground and belowground tissues, in this study, we focused only on the root metabolome of the control and the AMF-colonized roots as our primary aim was to capture the putative regulatory mechanisms in roots when colonized by AMF belonging to different genera. Some of the analyses for the Gigaspora treatment have less than six replicates as the root biomass was much lower than the other treatments.

### Shoot phosphorus content

The total P content in the leaves was analyzed by following the wet ashing procedure using inductively coupled plasma-mass spectrometry (ICP-MS). The leaf P concentration was converted to percent tissue P and then normalized with the SDW to obtain the P content (mg per shoot).

### Mycorrhizal percent colonization

A subsample of fine roots was placed in 70% ethanol for 24 h and then cleared with 10% potassium hydroxide (KOH) and stained with Trypan blue (Vierheilig *et al.*, 2005). The percent colonization was calculated based on the AMF structures (vesicles, arbuscules, hyphae) observed per intersection (McGonigle *et al.*, 1990). The roots were observed under a compound microscope for at least 100 intersections.

### DNA extraction and real-time quantitative polymerase chain reaction (qPCR) analysis

The main aim of conducting quantitative polymerase chain reaction (qPCR) was to compare the colonization of each AMF isolate in the different treatments. Quantitative PCR would also help to compare the colonization of *R. irregularis* in the Rhizophagus treatment and the MIX treatment, and similarly to compare the colonization of *G. gigantea* in the Gigaspora treatment and the MIX treatment. The DNA of *G. gigantea* and *R. irregularis* was extracted from the inoculum using Fast DNA spin kit for soil (mpbio.com). The extracted DNA was further used to optimize the primers for AMF species. Details are given in Methods S2; Table S1; Fig. S1.

### Mycorrhizal growth response (MGR)

The MGR was calculated as follows

$$\text{MGR}\,(\%) = \frac{\text{SDW (AM fungi} - \text{inoculated treatment)} - \text{mean SDW (non-inoculated treatment)}}{\text{mean SDW (non-inoculated treatment)}} \times 100$$

where SDW is the shoot dry weight. To evaluate the significance of MGR (%) of inoculated treatments from noninoculated treatment, a 95% confidence interval was calculated for all the treatments. A treatment was considered significant if the interval did not overlap with zero (Watts-Williams *et al.*, 2019). The mycorrhizal phosphorus response (MPR) was calculated by substituting SDW with P content (mg per shoot) in the equation.

### Metabolomics analysis

**Extraction** Root samples were initially ground to a fine powder using dry ice (−78°C). Next, 150 mg of the ground material was transferred to 2 ml tubes to which five ceramic beads and 1 ml of prechilled methanol were added. Details of extraction are given in Methods S3. The methanol–water phase was used for the analysis of primary and specialized metabolites (Suseela *et al.*, 2015, 2020).

**Analysis of primary metabolites** To analyze the primary metabolites, we transferred 100 µl of methanol–water phase to vials with glass inserts. To these inserts, we added 10 µl of ribitol in methanol (20 mg $l^{-1}$) and myristic acid in hexane (50 mg $l^{-1}$) as the internal standard and retention time lock, respectively, and the samples were then completely dried under nitrogen gas and derivatized (details are provided in Methods S3). An authentic standard mixture of 49 compounds of primary metabolites was derivatized as mentioned earlier and run along with the sample batch (Methods S3). The samples were analyzed using an Agilent 7980A GC (Agilent Technologies, Santa Clara, CA, USA) system coupled with 5975 C series quadrupole mass analyzer within 20 h after derivatization. The data was processed using MS Dial 3.0 (Lai *et al.*, 2018). Details of gas chromatography–mass spectrometry (GC–MS) parameters and data processing are provided in Methods S3.

**Analysis of specialized metabolites** The aliquot of the methanol–water phase was concentrated five times by drying under nitrogen gas and reconstituting in 50% methanol. The samples were analyzed using Ultimate 3000 high performance liquid chromatography (HPLC; Thermo Scientific, Waltham, MA, USA) coupled to an Orbitrap Fusion Tribrid mass spectrometer (Thermo Scientific) equipped with an electrospray ionization source operated in positive polarity (Suseela *et al.*, 2020). The data was processed using Compound Discoverer 3.1 (Thermo Scientific) that uses the proprietary algorithm for peak picking, merging, alignment, feature grouping, and searching online mass spectral libraries. Details of liquid chromatography–tandem mass spectrometry (LC-MS/MS) parameters, data processing, and identification of blumenols and flavonoids are provided in Methods S4.

### Statistical analyses

The effect of treatments on plant biomass, tissue P content, percent AMF colonization, and qPCR data was analyzed by using a two-way ANOVA with accessions and AMF treatments as the fixed factors. Both normality and equal variance assumptions were satisfied by log-transforming the data when necessary. The difference among treatments were assessed using Tukey's honestly significant difference (HSD) multiple comparisons test. We used JMP pro 14 for statistical analyses, and graphs were made using SigmaPlot v.14 (Systat Software Inc., Chicago, IL, USA). In the graphs, when the AMF treatment by sorghum accession interaction term was not significant, and only the main effect of a factor has a significant effect, we pooled the levels of the other factor. For the analysis of primary metabolites, the data were first normalized with ribitol (internal standard). Data (peak area) of primary and specialized metabolites were further normalized using log transformation and autoscaling to satisfy the assumptions of normality. Heatmaps were generated, and hierarchical clustering analysis was performed based on treatments and metabolites (MetaboAnalyst v.3.0; Xia & Wishart, 2016). The significant metabolites (false discovery rate (FDR) adjusted *P*-value < 0.05) were identified using a two-way ANOVA analysis followed by Tukey's HSD *post hoc* test. The data were further analyzed using partial least square- discriminant analysis (PLS-DA). The PLS-DA was followed by running a cross-validation model, which had a cross-validation $Q^2$ of > 0.8. For further validation, permutation testing was done to check the significance of class variations by setting permutation numbers at 2000, which was found to be significant with $P < 0.0005$.

## Results

### Plant benefits varied with AMF identity in single species inoculum and with plant genotype in mixtures

The roots of both sorghum accessions had > 65% AMF colonization in AMF inoculated treatments, whereas the NAM was devoid of AMF (Fig. 1a). Similarly, all AMF inoculated treatments had a similar percentage of arbuscules, while the NAM was devoid of arbuscules ($P < 0.001$; Fig. 1b). However, PI-562730 had a higher percentage of arbuscules than in PI-297130 ($P = 0.041$; Fig. 1c). The qPCR results showed no cross-species contamination in both Rhizophagus and Gigaspora treatments, and the NAM was devoid of both species. The mitochondrial DNA copies of *G. gigantea* were 44 times lower in the MIX treatment compared to the Gigaspora treatment ($P < 0.001$; Fig. 1d). The mitochondrial DNA copies of *R. irregularis* in the Rhizophagus treatment and the MIX treatment were similar ($P < 0.001$; Fig. 1d). Moreover, based on the mitochondrial DNA copy numbers of AMF species, PI-297130 accession had higher colonization of both *G. gigantea* and *R. irregularis* than PI-562730 accession ($P < 0.001$; Fig. 1e). Although the AMF percent colonization was similar in all AMF treatments, the mitochondrial DNA copies varied widely between *R. irregularis* and *G. gigantea*, which could be species-specific as indicated by Voříšková *et al.* (2017).

Although the AMF colonization was high in both sorghum accessions (Fig. 1), the growth and P uptake of the accessions varied with the identity of AMF in single inoculum treatments (Fig. 2). Both sorghum accessions subjected to the Rhizophagus treatment had the highest shoot dry biomass of all treatment groups.

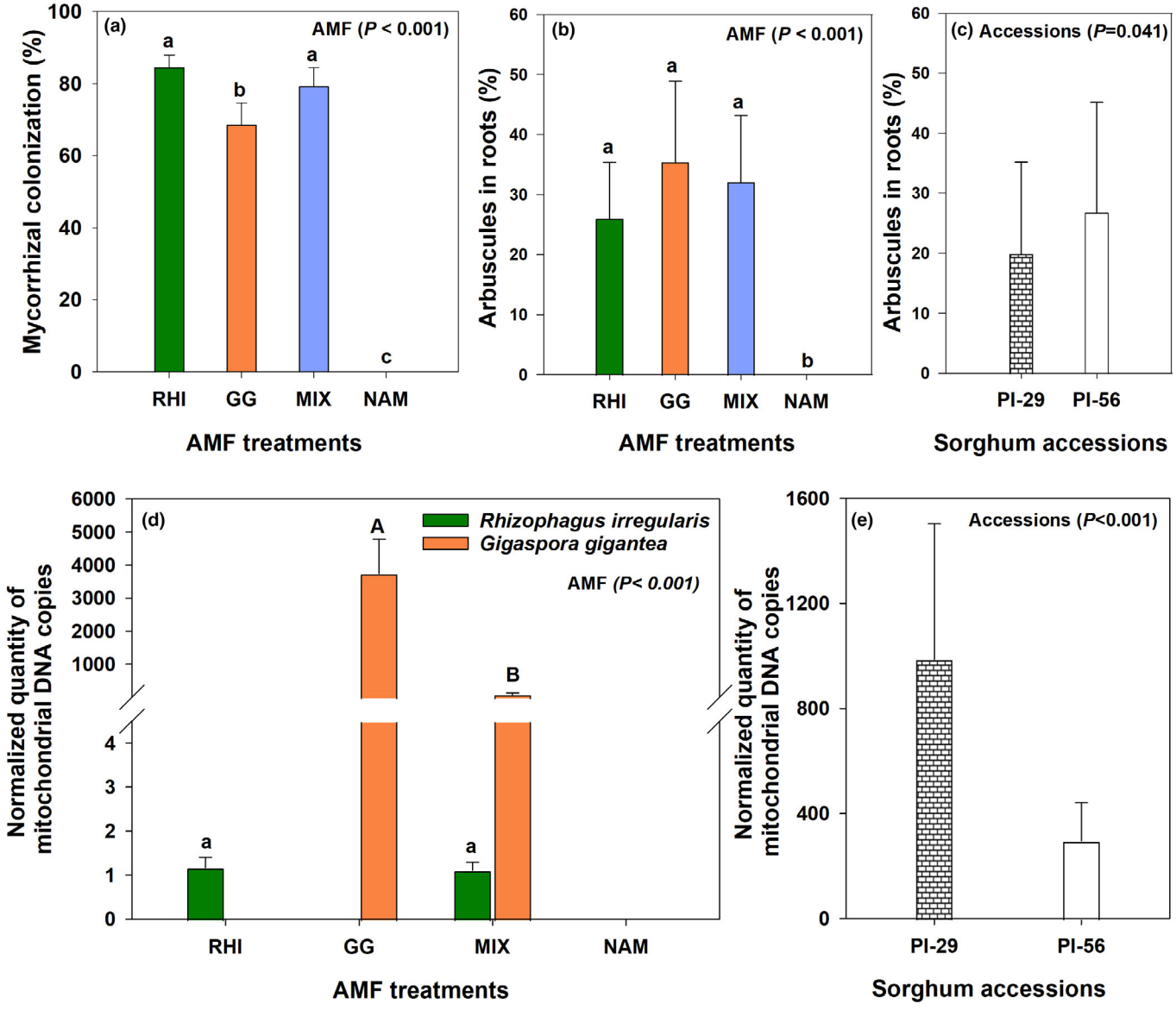


**Fig. 1** Percent mycorrhizal colonization (a), percent root intersections containing arbuscules (b, c), normalized quantity of mitochondrial DNA copies of arbuscular mycorrhizal fungi (AMF) (d, e) in the different treatments. Values represent mean ± SE, ($n$ = 6 for (a)–(c); $n$ = 3 (GG) to 6 (RHI, MIX, NAM) for (d) and (e)). Bars with different letters indicate a difference (Tukey's honestly significant difference (HSD)) between treatments. In (d) bars with different uppercase letters indicate a difference between (Tukey's HSD) mitochondrial DNA copies of GG and lowercase letters indicate a difference between (Tukey's HSD) mitochondrial DNA copies of RHI. Key: RHI, *Rhizophagus irregularis* (green); GG, *Gigaspora gigantea* (orange); MIX (1 : 1 of *Gigaspora gigantea* and *Rhizophagus irregularis;* blue); NAM, noninoculated control treatment (gray); PI-29, PI-297130; PI-56, PI-562730.

In the Rhizophagus treatment, the accessions PI-562730 and PI-297130 had 65% and 81% higher shoot dry biomass, respectively, compared to the NAM ($P < 0.001$; Fig. 2a). Both sorghum accessions in the Gigaspora treatment had 80% lower shoot biomass than the Rhizophagus treatment. In the MIX treatment, PI-297130 accession had approximately two times lower shoot biomass than PI-562730. As with the shoot dry biomass, the Rhizophagus treatment, and PI-562730 in the MIX treatment had the highest fresh root biomass, and Gigaspora treatment had the lowest root fresh biomass of all treatments (Fig. S2). In the MIX treatment, PI-297130 accession had 55% lower root fresh biomass than PI-562730. Furthermore, in the MIX treatment, PI-562730 had higher root fresh biomass than the NAM, while PI-297130 accession had similar biomass as the NAM (Fig. S2).

The shoot P content generally followed the same trend as the plant shoot dry biomass. The P content was highest in both accessions in the Rhizophagus treatment and PI-562730 in the MIX treatment, compared with the Gigaspora treatment and the NAM (Fig. 2b). The accessional difference in shoot P content was observed only in the MIX treatment where PI-297130 had 50% lower P content than PI-562730. Interestingly, PI-297130

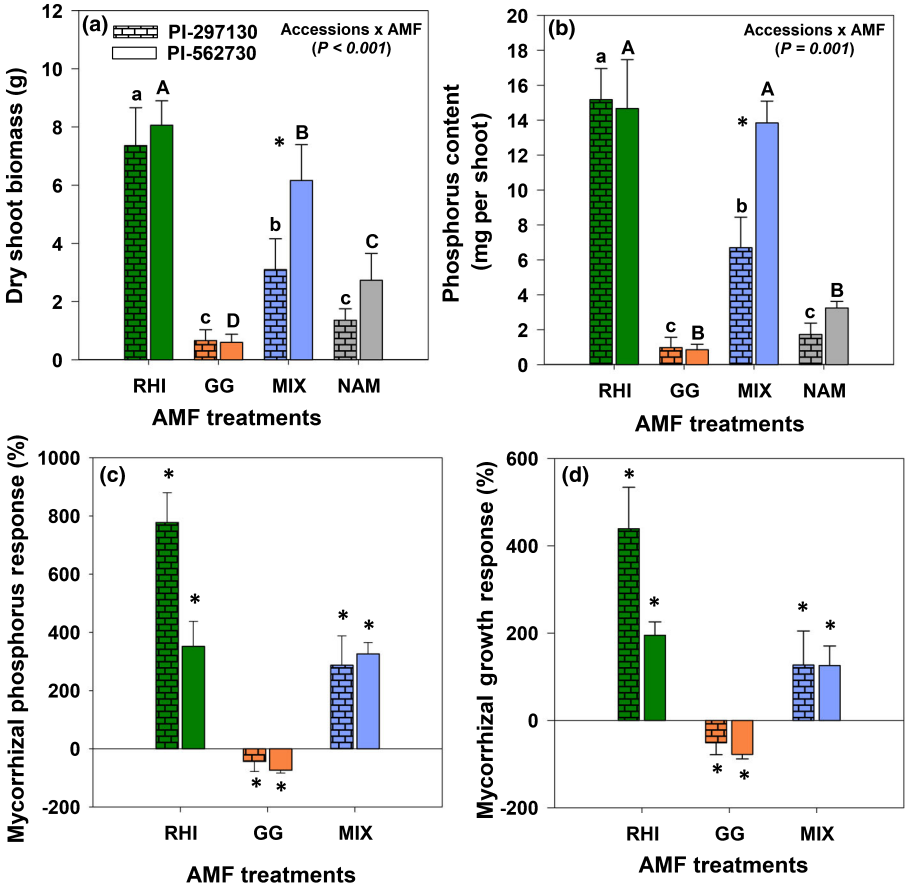


**Fig. 2** Dry shoot biomass (a), plant phosphorus content (b), mycorrhizal phosphorus response (c; MPR) and mycorrhizal growth response (d; MGR) of two sorghum genotypes – PI-297130 and PI-562730 inoculated with arbuscular mycorrhizal fungi (AMF) treatments. Values represent mean ± SE ($n$ = 6). Bars with different uppercase letters indicate a difference (Tukey's honestly significant difference (HSD)) between PI-562730 along AMF treatments and lowercase letters indicate a difference (Tukey's HSD) between PI-297130 along AMF treatments. Asterisks in (a) and (b) indicate a difference between accessions within an AMF treatment ($P < 0.05$). Asterisks in (c) and (d) indicate that the AMF treatment is different from the control at 95% confidence interval. Key: RHI, *Rhizophagus irregularis* (green); GG, *Gigaspora gigantea* (orange); MIX (1 : 1 of *Gigaspora gigantea* and *Rhizophagus irregularis;* blue); NAM, noninoculated control treatment (gray).

had higher P content in the Rhizophagus treatment compared with the MIX treatment resulting in a similar trend with MPR (Fig. 2c). However, unlike PI-297130, the accession PI-562730 had similar MPR in both Rhizophagus and MIX treatments corresponding to a similar shoot P content in both these treatments (Fig. 2c). Only in the Rhizophagus treatment, PI-297130 had higher MPR and MGR than PI-562730 (Fig. 2c,d). In the Gigaspora treatment, irrespective of the higher colonization, the contribution of *G. gigantea* to plant growth or P acquisition was negligible and similar to the NAM, except for the biomass of PI-562730 in the Gigaspora treatment (Fig. 2a,b). Thus, the percent MGR varied from being positive with Rhizophagus and MIX treatments to negative with *G. gigantea* irrespective of the sorghum accessions (Fig. 2d).

## Root metabolome mirrored the mycorrhizal symbiotic phenotype

Among the 35 root primary metabolites detected and annotated using GC–MS and MS-dial 3.0, respectively (Table S2), the abundance of 32 metabolites was significantly different (FDR adjusted $P$-value < 0.05) between AMF treatments. The heatmap (Fig. 3) and PLS-DA (Fig. S3) of the primary metabolites revealed that the Rhizophagus and MIX treatments clustered differently from the Gigaspora treatment and the NAM with both principal components together explaining a variation of 74.1% in the data. The Rhizophagus and MIX treatments, which exhibited a mutualistic phenotype, had a higher abundance of organic acids. In contrast, the Gigaspora treatment, which exhibited a parasitic phenotype, had a higher abundance of amino acids (Fig. 3).

The roots from the Gigaspora treatment and the NAM were abundant in amino acids ($P < 0.001$; Fig. 4a) compared with other treatments. The accession PI-297130 had a higher abundance of total amino acids than PI-562730 ($P = 0.001$, Fig. 4b). Among the 18 amino acids that were identified, 12 of them (aspartic acid, β-alanine, γ-aminobutyric acid (GABA), glutamine, glycine, isoleucine, leucine, lysine, ornithine, threonine, tyrosine, valine) had higher abundance in Gigaspora treatment compared to all other treatments across the two accessions ($P < 0.05$; Figs 3, S4; Table S2). The abundance of β-cyanoalanine and asparagine was higher in the Gigaspora treatment and NAM compared with the Rhizophagus and MIX treatments ($P < 0.001$; Fig. 4c,d) potentially from the cyanide detoxification pathway (Fig. 4e). Although most of the amino acids were higher in the Gigaspora treatment than the NAM, the relative abundance of asparagine was highest among all the identified amino acids (Fig. S4) leading to a similar abundance of total amino acids in the Gigaspora treatment and the NAM. Glutamic acid, histidine, and serine ($P < 0.001$; Figs 3, S4) were more abundant in all AMF inoculated treatments than the NAM. Other nitrogenous compounds such as urea and allantoic acid had a higher concentration in Gigaspora treatment and the NAM than

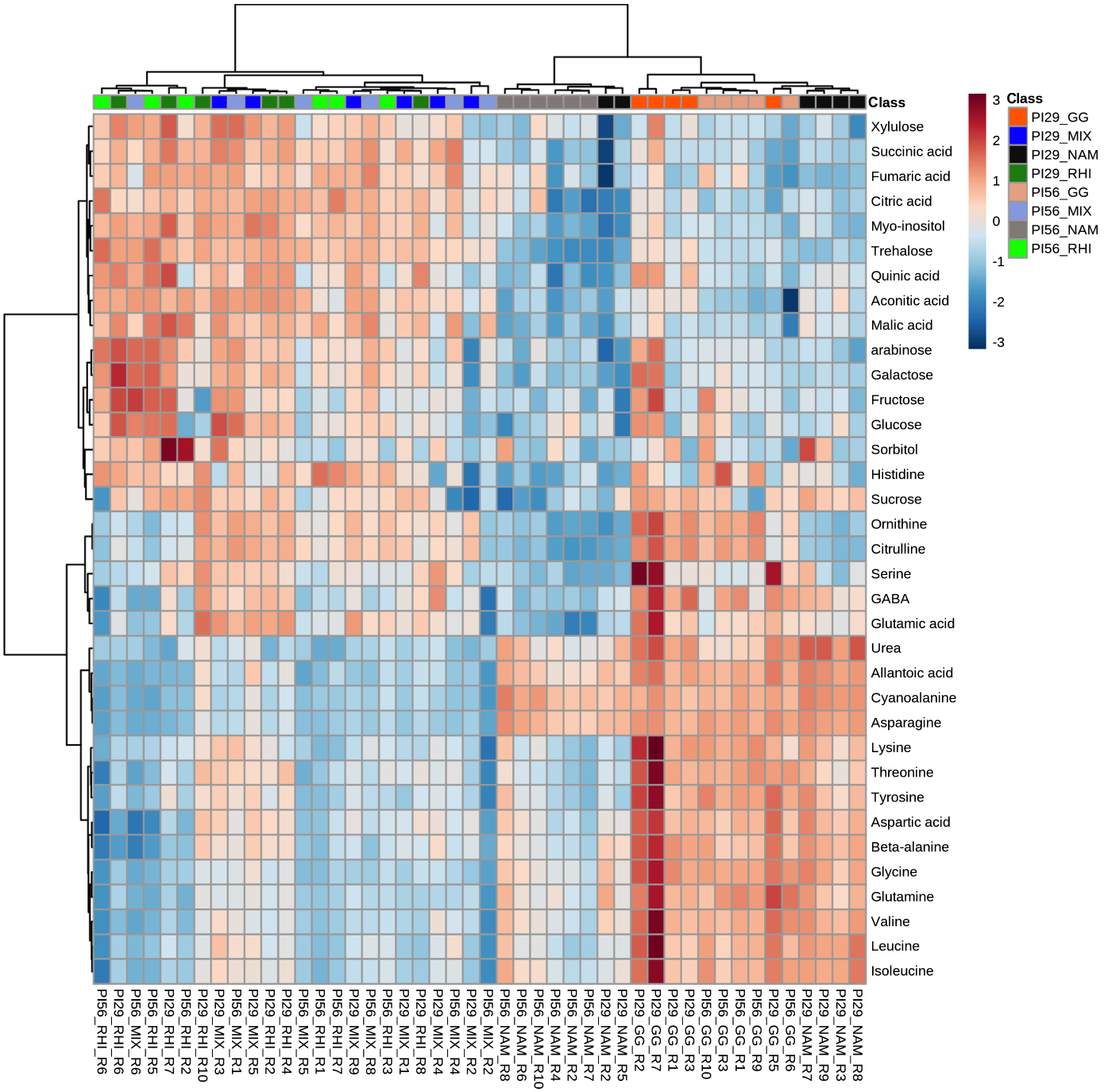


**Fig. 3** Heatmap and two-way hierarchical clustering of the intensities of 35 primary metabolites of the control and arbuscular mycorrhizal fungi (AMF)-colonized roots of two sorghum genotypes – PI-297130 (PI-29) and PI-562730 (PI-56) exposed to different AMF treatments – *Rhizophagus irregularis* (RHI), *Gigaspora gigantea* (GG), 1 : 1 of *Gigaspora gigantea* and *Rhizophagus irregularis* (MIX) and the noninoculated control treatment (NAM). Each column represents a replicate from a treatment, and each row represents a metabolite. Blue to red color in the scale represents an increase in the abundance of a metabolite.

Rhizophagus and MIX treatments ($P < 0.001$; Figs 3, S4). Additionally, among the accessions, urea and allantoic acid were more abundant in PI-297130 genotype than the PI-562730 genotype ($P < 0.001$).

Sugars and sugar alcohols were more abundant in the roots of AMF inoculated treatments (Rhizophagus, Gigaspora, and MIX treatments) than the NAM (Fig. 3). The total sugars were higher in the mycorrhizal treatments than the NAM ($P < 0.001$, Fig. S5a) and were higher in PI-297130 accession compared to PI-562730 accession (Fig. S5b). Detailed results of sugars are provided in Notes S1. Among the sugar alcohols identified, myo-inositol varied with the AMF treatments where

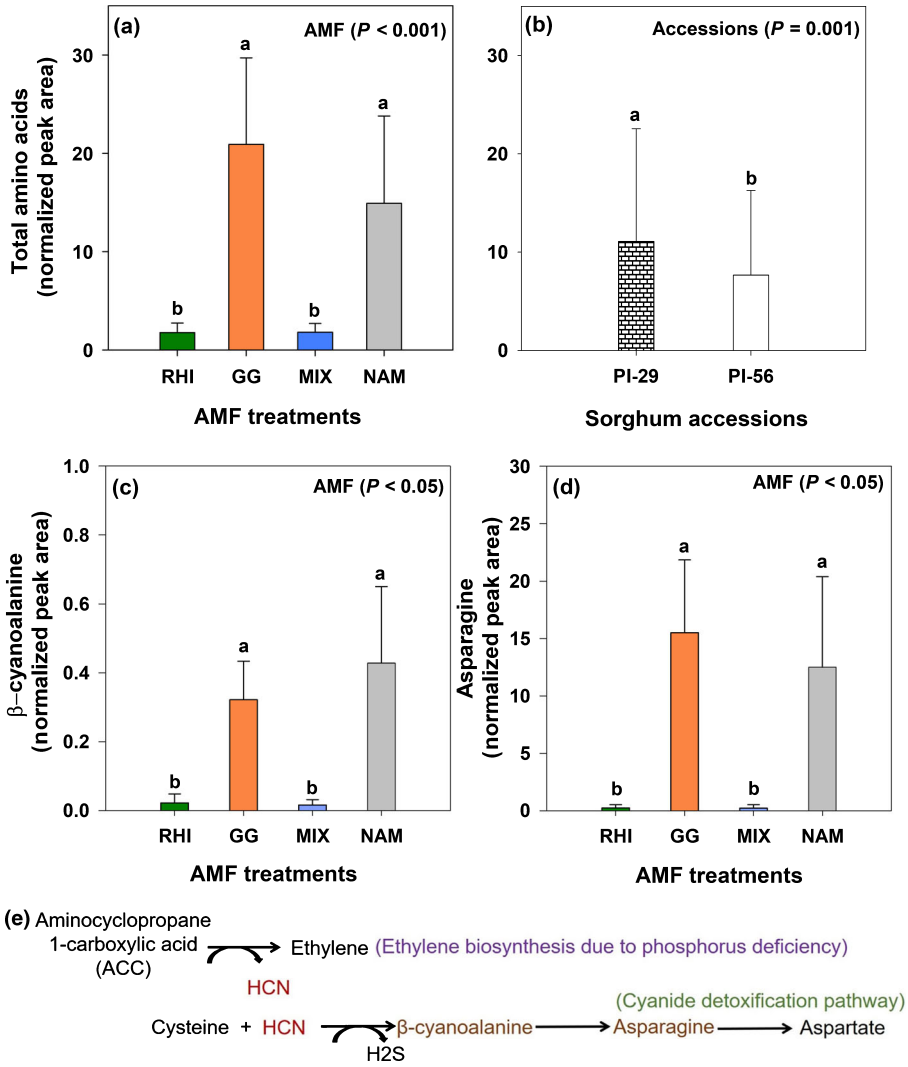


**Fig. 4** Normalized peak area of total amino acids (a, b) β-cyanoalanine (c), and asparagine (d) of the control and arbuscular mycorrhizal fungi (AMF)-colonized roots of two sorghum genotypes – PI-297130 and PI-562730. Values represent mean ± SE ($n = 5$ for GG; $n = 6$ for all other treatments). Bars with different letters indicate a difference (Tukey's honestly significant difference (HSD)) between treatments. (e) Representation of the cyanide detoxification pathway. Key: RHI, *Rhizophagus irregularis* (green); GG, *Gigaspora gigantea* (orange); MIX (1 : 1 of *Gigaspora gigantea* and *Rhizophagus irregularis;* blue); NAM, noninoculated control treatment (gray); PI-29, PI-297130; PI-56, PI-562730.

Rhizophagus and MIX treatments, which exhibited a mutualistic phenotype, had higher abundance of myo-inositol than Gigaspora treatment and NAM ($P < 0.0001$; Fig. S4).

A total of five organic acids were identified in the roots of both sorghum accessions, which varied with the AMF treatments and showed an opposite trend as that of amino acids (Figs 3, S5c). All identified organic acids (fumaric acid, aconitic acid, succinic acid, malic acid and citric acid; Table S2) were higher in Rhizophagus and MIX treatments than the Gigaspora treatment and NAM ($P < 0.0001$; Figs 3, S4). Thus, the total organic acids were higher in Rhizophagus and MIX treatments compared to other treatments (Fig. S5c).

Similar to the primary metabolome, the specialized metabolome in the roots also mirrored the mutualistic or parasitic phenotype of the AMF symbiosis. After accounting for the multiple adduct formation, 3566 metabolic features of specialized metabolites were detected (signal-to-noise of 5 : 1) in the roots of different AMF treatments. Based on the 3566 metabolic features, the Gigaspora treatment and NAM clustered separately from the Rhizophagus and MIX treatments as visualized by the heat map (Fig. S6) and PLS-DA (Fig. S7). The specialized metabolic features expressed in different AMF treatments were grouped into six distinct clusters (Fig. S6). Cluster I represented the specialized metabolic features that were higher only in the Rhizophagus and MIX treatments, which exhibited a mutualistic phenotype. Cluster II contained metabolic features that were abundant in all AMF inoculated treatments. Clusters III and IV comprised the metabolic features inherent to PI-562730 and PI-297130 accessions, respectively. Cluster V included those metabolic features abundant only in plants that experienced P limitation (Gigaspora treatment and NAM), and cluster VI included those specialized metabolic features that were higher only in the Gigaspora treatment. The top 100 significant specialized metabolic features expressed in different AMF treatments also followed the same trend where the Gigaspora treatment and NAM clustered separately from the Rhizophagus and MIX treatments and these top 100 specialized metabolic features belonged to clusters I–IV (Fig. 5).

Tyrosine (Fig. 6a) and an intermediate compound in the production of dhurrin, namely *p*-hydroxyphenylacetaldoxime (Fig. S8a; Table S3) with antifungal properties was more abundant in the Gigaspora treatment compared to all other treatments ($P <$

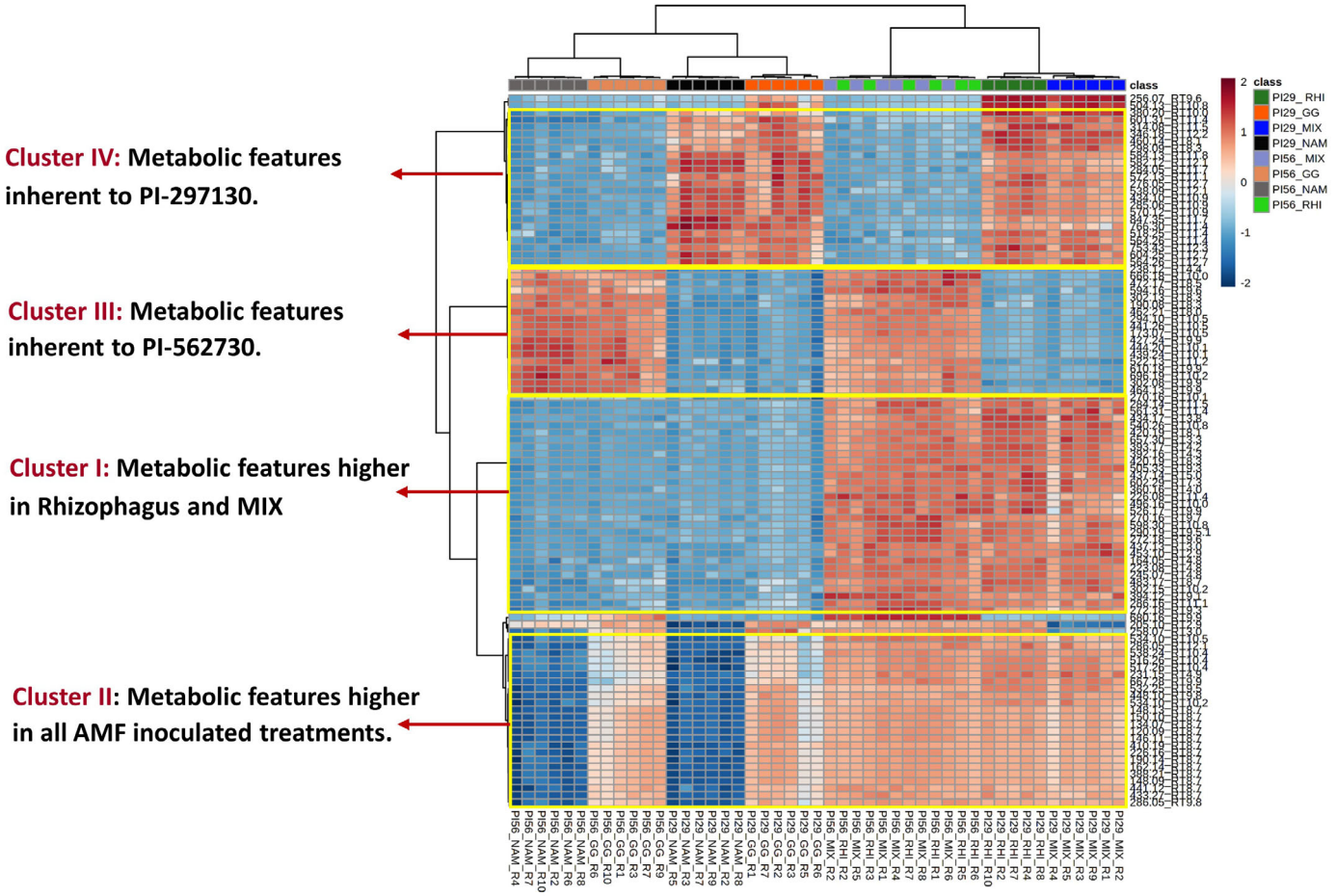


**Fig. 5** Heatmap and two-way hierarchical clustering of the intensities of metabolic features of specialized metabolites of the control and arbuscular mycorrhizal fungi (AMF)-colonized roots of two sorghum genotypes – PI-297130 (PI-29) and PI-562730 (PI-56) exposed to different AMF treatments – *Rhizophagus irregularis* (RHI), *Gigaspora gigantea* (GG), 1 : 1 of *Gigaspora gigantea* and *Rhizophagus irregularis* (MIX) and the noninoculated control treatment (NAM). Each column represents a replicate from a treatment, and each row represents a metabolic feature. Blue to red color in the scale represents an increase in the abundance of a metabolic feature. The heatmap represents the top 100 metabolic features that showed the strongest differences according to ANOVA from 3566 metabolic features of specialized metabolites.

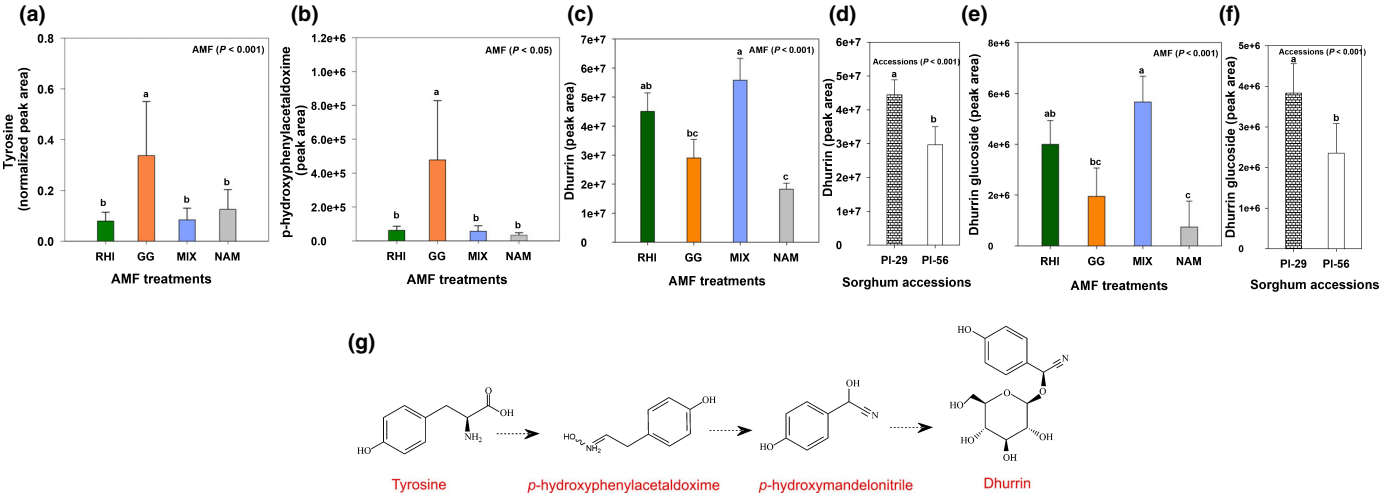


**Fig. 6** Peak area of (a) tyrosine, (b) *p*-hydroxyphenylacetaldoxime, (c–f) dhurrin in the control and arbuscular mycorrhizal fungi (AMF)-colonized roots of two sorghum genotypes – PI-297130 and PI-562730 and (g) representation of dhurrin synthesis pathway. Values represent mean ± SE ($n = 6$). Bars with different letters indicate a difference (Tukey's honestly significant difference (HSD)) between treatments. Key: RHI, *Rhizophagus irregularis* (green); GG, *Gigaspora gigantea* (orange); MIX (1 : 1 of *Gigaspora gigantea* and *Rhizophagus irregularis;* blue); NAM, noninoculated control treatment (gray); PI-29, PI-297130; PI-56, PI-562730.

0.05; Fig. 6b). We also identified dhurrin (Fig. S8b), which had a higher abundance in Rhizophagus and MIX treatments compared to NAM, whereas Gigaspora treatment was neither different from Rhizophagus treatment nor from NAM ($P < 0.001$; Fig. 6c–f). The total blumenols in Rhizophagus and MIX treatments were approximately twice that of Gigaspora treatment ($P < 0.001$; Figs S9, S10), and there was no accessional difference. Details of blumenols (Table S4; Fig. S11) and flavonoids (Figs S12, S13) are given in Notes S1. Among the flavonoids, luteolin–kaempferol with acetyl hexose–oxalyl pentose moiety was abundant only in the Gigaspora treatment (Figs 7, S14; Table S3).

## Discussion

Our results revealed that the identity of the AMF species and plant accessions exerted differential regulation on the outcome of the symbiosis. Moreover, rather than retaining the plant identity, both the primary and specialized metabolites in the roots of the sorghum accessions mirrored the mutualistic/parasitic AMF phenotype. While sugars were abundant in all AMF treatments indicating a carbon sink to sustain AMF, the organic acids were higher in Rhizophagus and MIX treatments with higher P uptake and increased biomass. Contrastingly, the amino acids were higher in the Gigaspora treatment and the NAM, potentially indicating the stress experienced by the plants. Specialized metabolites have a critical role in establishing a functional AMF–plant symbiosis. In our study, the specialized metabolites were higher in the Rhizophagus and MIX treatments reflecting a successful modulation of the plant defense during the symbiosis that could partly contribute to a mutualistic phenotype. However, the symbiosis with *G. gigantea* that exhibited a parasitic phenotype resulted in the abundance of an antifungal compound, *p*-hydroxyphenylacetaldoxime, in both sorghum accessions. Thus, our results for the first time revealed that the plant–AMF symbiosis that exhibits a parasitic phenotype could elicit antagonistic defense responses in the host plant irrespective of the host genotype. Despite producing the antifungal compound, the plant could not sanction

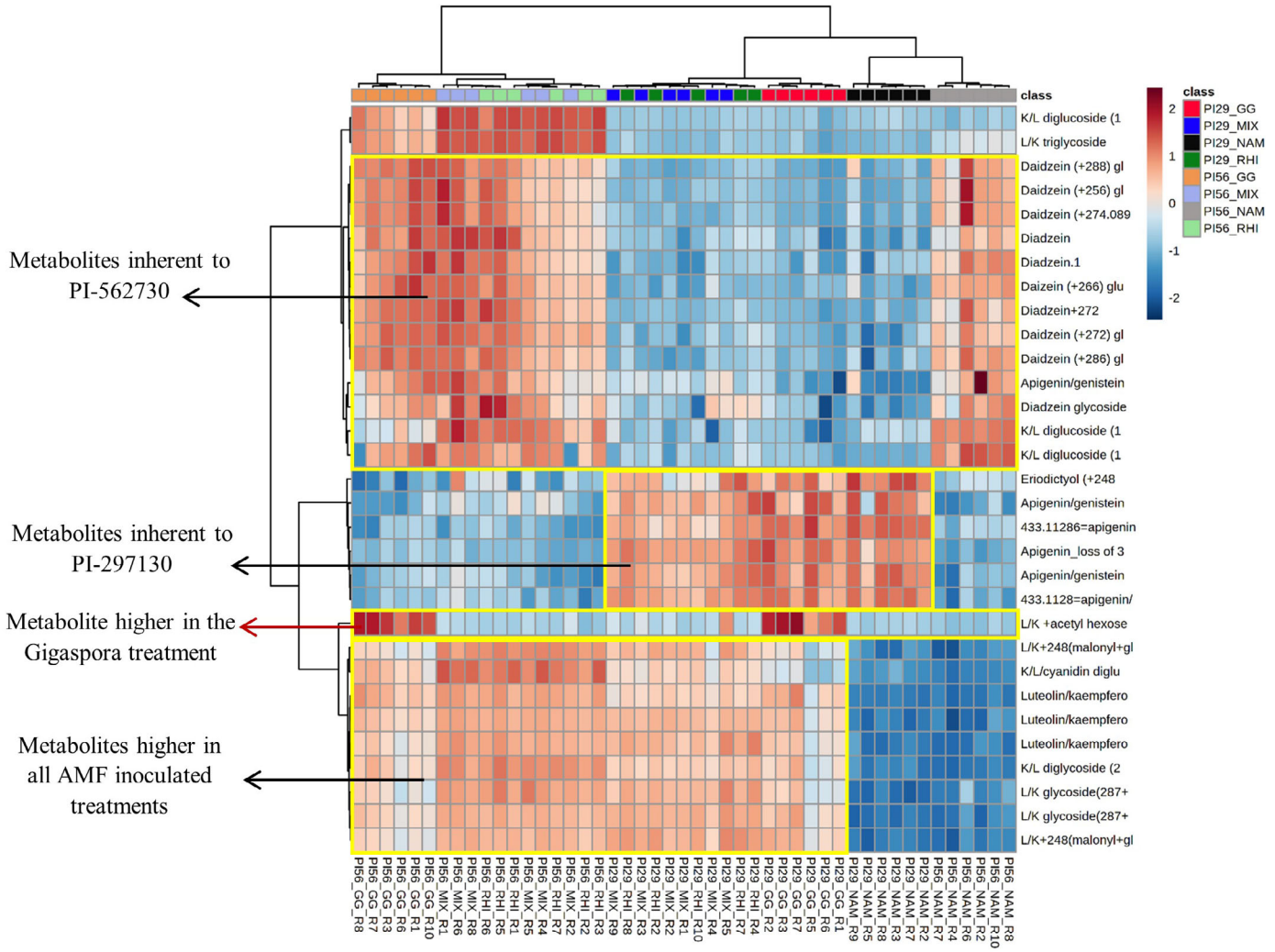


**Fig. 7** Heatmap of the intensities of 31 flavonoid aglycones and glycosides of two sorghum genotypes – PI-297130 (PI-29) and PI-562730 (PI-56) in the control and arbuscular mycorrhizal fungi (AMF)-colonized roots. Key: RHI, *Rhizophagus irregularis*; GG, *Gigaspora gigantea*; MIX (1 : 1 of *Gigaspora gigantea* and *Rhizophagus irregularis*); NAM, noninoculated control treatment; K/L, kaempferol–luteolin.

*G. gigantea* that exhibited the parasitic phenotype. However, a mutualistic symbiotic association prevailed when the host plants were exposed to a mixture of AMF, thus stabilizing the plant–AMF symbiosis.

Although the partner specificity in the plant–AMF association is low, it is well known that there is high specificity in the outcome of this association. The outcome can vary with the identity of the plant, AMF, and the environment (Berruti *et al.*, 2016; Hazard & Johnson, 2018), and there is high functional diversity of AMF in the provision of growth benefits or tolerance to biotic and abiotic stress (Allen *et al.*, 1995; Klironomos, 2000; Lee *et al.*, 2013). In our study, there was high percent colonization and mitochondrial DNA copies of AMF in all AMF treatments in both sorghum accessions. Thus, similar to previous studies our results also indicated less host–AMF specificity at the level of AMF colonization. However, the outcome of this symbiotic association varied with the identity of the AMF in single species inoculation treatments. The mutualistic phenotype, as visualized by the increased biomass and P uptake, was evident in the Rhizophagus treatment in both sorghum accessions. However, the symbiosis with *G. gigantea* exhibited a parasitic phenotype, which resulted in a negative MGR. The higher AMF colonization and higher relative abundance of sugars in the Gigaspora treatment compared to NAM indicated a potential carbon drain from the host plant while providing no P in return. In AMF colonized plants, the mycorrhizal pathway of P uptake dominates over the direct pathway of P uptake by plants (Smith & Smith, 2011). However, if the AMF withholds the provision of P via the AMF pathway (Lendenmann *et al.*, 2011), it results in P deprivation and growth depression in plants.

In plant–AMF symbiosis, both plants and AMF species can discriminate between the different partners and allocate more resources to the most efficient partner (Helgason *et al.*, 2002; Kiers & Van Der Heijden, 2006; Bever *et al.*, 2009; Kiers *et al.*, 2011). Partner choice is thus an important mechanism that stabilizes plant–AMF mutualism (Kiers *et al.*, 2011; Walder & Van Der Heijden, 2015). The plant could preferentially provide more carbon to the beneficial AMF partner reducing the abundance of the lower-quality partner (Kummel & Salant, 2006; Werner *et al.*, 2018). However, fungal competition and differences in colonization kinetics between the two AMF species could also be major factors that regulate the colonization of different AMF species in a mixture. In the MIX treatment, *G. gigantea* could have been partly inhibited due to potential competition from *R. irregularis*. Previous studies have also reported that members of Glomeraceae colonized roots faster and had higher percent colonization than isolates from Gigasporaceae, which are slow colonizers (Hart & Reader, 2002; Blažková *et al.*, 2021). Thus, the colonization of *G. gigantea* was lower in the MIX treatment compared to the Gigaspora-alone treatment, while *R. irregularis* had similar colonization in the Rhizophagus-alone and the MIX treatment.

Interestingly, in the MIX treatment, the host identity was important in determining the outcome (growth and P uptake) of this symbiosis where PI-297130 had lower P uptake and biomass than PI-562730. A potential reason for this decrease in biomass of PI-297130 only in the MIX treatment could be the inability of PI-297130 to control carbon transfer to the less cooperative fungal partner when inoculated with a mixture of AMF species. Any additional carbon supply to *G. gigantea* in the MIX treatment, which did not contribute to P uptake, could lower the biomass (Berger & Gutjahr, 2021). Previous research has indicated that despite the lack of P uptake through the AMF pathway, the plant inoculated with *Gigaspora rosea* showed growth depression potentially due to the higher demand for carbon from the plant compared to *Glomus intraradices* (Smith *et al.*, 2003; Lendenmann *et al.*, 2011). Our results suggest that under high AMF diversity that occurs under natural field settings, different plant genotypes/species may vary in their capacity to promote the most cooperative AMF, thus altering the outcome of this symbiotic association. It should also be noted that although in our study *G. gigantea* exhibited a parasitic phenotype, it could exhibit a mutualistic phenotype with other plant species or environmental conditions. For example, in Watts-Williams *et al.* (2019), PI-562730 exhibited a positive MGR with *G. gigantea* where the P source was calcium hydrogen phosphate ($CaHPO_4$). However, in our study, we observed a negative MGR with *G. gigantea* where the P source was $FePO_4$. A previous study has reported that the benefit of AMF to host plant could depend not only on P availability but also the form of P (Reynolds *et al.*, 2006). Based on MGR and MPR, the host identity was important only in the Rhizophagus treatment where PI-297130 had greater MGR and MPR than PI-562730, which may be partly due to the difference in AMF colonization and the greater dependency of PI-297130 to *R. irregularis* for P uptake and growth compared to PI-562730.

The reprogramming of the root metabolome also varied strictly with the mutualistic or parasitic phenotype of the AMF symbiosis. The abundance of sugars did not vary with AMF treatments, while amino acids, and organic acids were differentially modulated in different AMF treatments. In plant–AMF symbiosis, the plant reciprocates the nutritional benefits (mainly P) by AMF by providing sugars and fatty acids to AMF (Schweiger & Müller, 2015; Jiang *et al.*, 2017). Total sugars were higher in all AMF treatments than the NAM, indicating that both AMF species created a carbon sink in the roots. The higher abundance of sugars even in the Gigaspora treatment where the plants exhibited growth depression indicates a potential carbon drain from the plant (Lerat *et al.*, 2003; Smith *et al.*, 2003). The higher abundance of trehalose in Rhizophagus and MIX treatments can be linked to a functional mycorrhizal symbiosis (Lohse *et al.*, 2005). The polyols such as myo-inositol also had higher abundance in Rhizophagus and MIX treatments, which exhibited a mutualistic phenotype. The abundance of polyols may help the plants to reduce the hexose gradient at the root–fungal interface to regulate the sugar supply to AMF (Nehls & Bodendiek, 2012).

Unlike sugars, organic acids were abundant in the Rhizophagus and MIX treatments that experienced less P stress compared to the Gigaspora treatment and the NAM. The activation of the tricarboxylic acid (TCA) cycle in the mitochondria of mycorrhizal roots would provide the adenosine triphosphate (ATP) and carbon skeletons that are necessary for the production of fatty acids and amino acids may indicate a functional symbiosis (Lohse

*et al.*, 2005). Plants under P stress release organic acids to solubilize the unavailable P in the soil (Hernández *et al.*, 2007; Shen *et al.*, 2011). However, previous studies have indicated that the amount of carboxylates in the rhizosphere decreased when the plants were colonized with AMF (Ryan *et al.*, 2012; Nazeri *et al.*, 2014). This could be due to the release of organic acids by AMF through hyphal exudates for the uptake of P (Tawaraya *et al.*, 2006; Andrino *et al.*, 2021). As we have not measured the rhizosphere carboxylates, we speculate that the higher abundance of organic acids in roots of AMF treatments that exhibited a mutualistic phenotype could be potentially because AMF managed to uptake P by utilizing the hyphal exudates.

We observed a major shift in the abundance of amino acids with mycorrhizal phenotype and treatments that experienced P limitation such as the Gigaspora treatment and the NAM. Phosphorus stress upregulates the production of ethylene that affects root architecture, including root extension (Lynch & Brown, 1997; Borch *et al.*, 1999), to help the plant to explore more soil to obtain P (Ma *et al.*, 2003). Among all the amino acids identified, asparagine and β-cyanoalanine were higher in both Gigaspora treatment and the NAM where the plants experienced P deficiency. This could result from the upregulation of the ethylene biosynthesis pathway that leads to the production of hydrogen cyanide (HCN; Wang *et al.*, 2002), which is further detoxified by the conversion of HCN to β-cyanoalanine (Garciá *et al.*, 2010) and then to asparagine (Fig. 4e). Moreover, the abundance of many amino acids in Gigaspora treatment could be due to the lack of utilization of the amino acids in downstream pathways or the degradation of proteins. Previous studies have reported that AMF symbiosis with a mutualistic phenotype had lower concentrations of amino acids, particularly the aromatic amino acids that contribute to the production of several specialized metabolites (Schweiger *et al.*, 2014; Rivero *et al.*, 2015). The concentration of specialized metabolites was lower in the Gigaspora treatment that exhibited a parasitic phenotype compared with the Rhizophagus and MIX treatments that exhibited a mutualistic phenotype.

One of the novel findings of our study was the higher abundance of an oxime with antifungal properties in the Gigaspora treatment, which exhibited the parasitic phenotype. This oxime, namely *p*-hydroxyphenylacetaldoxime, is an intermediary in the production of dhurrin (Fig. 6g). Dhurrin, the cyanogenic glycoside in sorghum implicated in herbivore defense, is produced from the amino acid tyrosine. Upon herbivore attack, dhurrin is converted into HCN, which is toxic to herbivores. The efficiency of dhurrin production is governed by a metabolon formation (Laursen *et al.*, 2016), which comprises a multienzyme complex that catalyzes successive reactions (Neilson *et al.*, 2013). However, the disassembly of this metabolon potentially due to the release of reactive oxygen species (ROS) following a fungal attack would result in the production of *p*-hydroxyphenylacetaldoxime with antifungal properties (Moller, 2010; Sørensen *et al.*, 2018). Oximes are used as chemical fungicides as they are generally toxic to fungi (Drumm *et al.*, 1995) and can be converted to highly reactive nitroso compounds resulting in protein conjugation products signaling an immune response from the plant (Moller, 2010). In our study, the amino acid tyrosine was abundant in the Gigaspora treatment subsequently leading to the higher production of *p*-hydroxyphenylacetaldoxime.

As plants initially perceive all microorganisms as putative pathogens, the nonself-molecules from these microorganisms trigger an immune response and activate a defense program in plants during the initial stages of colonization (Liu *et al.*, 2003; Zamioudis & Pieterse, 2012). Subsequently, the host plant and the AMF participate in a coordinated molecular dialog which would then suppress the immune response of the host plant (Zamioudis & Pieterse, 2012) to enable the AMF to colonize the plant successfully. Although *G. gigantea* successfully colonized the plant, the production of this antifungal compound suggests that after colonization, the plant potentially perceived *G. gigantea* as a fungal invader and upregulated the defense since the AMF was providing a limited return for the carbon investment. Similarly, the acetyl glycoside of kaempferol–luteolin was found to be higher only in the Gigaspora treatment. Acylated flavonoids are effective against ultraviolet (UV) radiations and are considered as UV absorbers (Skaltsa *et al.*, 1994; Tohge *et al.*, 2015). Acylated flavonoids are implicated to have a functional role in biotic interactions with microorganisms (Alseekh *et al.*, 2020). The same properties that protect the plant from oxidizing radiation may also protect it from pathogens. Thus, the production of the acetyl glycoside of kaempferol–luteolin could also be an antagonistic defense response against *G. gigantea,* which exhibited a parasitic phenotype. However, even with the production of these antagonistic defense compounds the plants were not able to sanction *G. gigantea*.

Although both sorghum accessions had unique specialized metabolites inherent to each accession, the abundance of a majority of specialized metabolites and the clustering of the treatments (Fig. 5) was driven more by the identity of the AMF than by the identity of the sorghum accessions. The specialized metabolites had higher abundance in the Rhizophagus and MIX treatments that exhibited positive growth benefits due to the AMF symbiosis. Furthermore, AMF primes the plant for better defense against biotic and abiotic stresses mainly through the upregulation of specialized metabolites (Pozo & Azcón-Aguilar, 2007; Kaur & Suseela, 2020). The successful modulation of specialized metabolites can contribute to a functional plant–AMF symbiosis as observed in the Rhizophagus and MIX treatments. Two important specialized metabolite pathways that were upregulated in these treatments were the phenylpropanoid and carotenoid (apocarotenoid) pathways that led to the production of several specialized metabolites, including flavonoids and blumenols, respectively. Flavonoids play a critical role in plant–AMF interactions by facilitating spore germination, hyphal growth and differentiation, the formation of entry points, and root colonization, and thus the abundance can increase under plant–AMF symbiosis (Vierheilig *et al.*, 1998; Aseel *et al.*, 2019). AMF is also reported to increase specialized metabolites such as blumenols which serve as the biomarker for AMF symbiosis (Hill *et al.*, 2018; Wang *et al.*, 2018). It should be noted that since the AMF-colonized roots had fungal material, the root metabolome measured in this study would be a mixture of both plant and fungal-derived metabolites (Rivero *et al.*, 2015; Hill *et al.*, 2018). This is particularly true with primary metabolites where the fungal sugar trehalose can

contribute to total sugars. There could also be AMF genera/species-specific fine-tuning of many plant or fungal-derived metabolites (Schweiger *et al.*, 2014; Rivero *et al.*, 2015). Moreover, as roots are the immediate site of AMF association, the root metabolome could be different from the systemic metabolic effects of AMF on leaves.

Our study provides novel insights into the metabolic reprogramming in mycorrhizal roots that ensures a mutualistic or parasitic phenotype following plant–AMF symbiosis. Overall, our results revealed that AMF identity could alter the plant growth response, P uptake, and metabolome uniquely depending on the AMF symbiotic phenotype. Although the plants could not sanction the AMF species that resulted in a parasitic phenotype, in the presence of mixtures of AMF species, plants do discriminate against the AMF species that result in parasitic phenotype, potentially through a combination of fungal competition and carbon allocation by host plants. Thus, conditions that promote greater diversity of AMF in agricultural soils would enhance the association of plants with favorable fungal partners. Our study also emphasizes the importance of the differential ability of plant genotypes in selecting and rewarding the most cooperative AMF partner. This underscores the need to further explore breeding for genotypes that can select cooperative AMF partners, which results in a better outcome of the plant–AMF symbiotic association.

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## Author contributions

VS conceptualized and designed the study. SK conducted the experiment and data analyses. BJC helped SK in performing qPCR analysis. SK wrote the draft of the manuscript with input from VS and all authors contributed to revisions.

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## Data availability

The authors declare that all data are available from the corresponding author upon request.

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## Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Fig. S1** Standard curves generated using plasmid DNA involving different primer inserts.

**Fig. S2** Fresh root biomass of two sorghum genotypes, PI-297130 and PI-562730 inoculated with different AMF treatments.

**Fig. S3** PLS-DA of primary metabolites from roots of sorghum genotypes inoculated with different AMF treatments.

**Fig. S4** Response of individual metabolites to different AMF treatments in representative metabolic pathways.

**Fig. S5** Normalized content of total sugars and organic acids in different AMF treatments and sorghum accessions.

**Fig. S6** Heatmap and two-way hierarchical clustering of the intensities of 3566 metabolic features of specialized metabolites from roots of sorghum accessions inoculated with different AMF treatments.

**Fig. S7** PLS-DA of the intensities of 3566 metabolic features of specialized metabolites from roots of sorghum accessions inoculated with different AMF treatments

**Fig. S8** Observed MS/MS fragmentation spectra of tentatively identified *p*-hydroxyphenylacetaldoxime and dhurrin.

**Fig. S9** Observed MS/MS fragmentation spectra of glycoside derivatives of blumenol C and hydroxyblumenol C.

**Fig. S10** Total blumenols obtained from roots of sorghum genotypes inoculated with different AMF treatments.

**Fig. S11** Heatmap and two-way hierarchical clustering of the intensities of 10 blumenol glycosides from roots of sorghum accessions inoculated with different AMF treatments.

**Fig. S12** PLS-DA of the intensities of 31 flavonoid aglycones and their glycosides obtained from roots of sorghum accessions inoculated with different AMF treatments.

**Fig. S13** Total flavonoids in sorghum accessions: PI-297130 and PI-562730.

**Fig. S14** Peak area of acetyl glycoside of kaempferol–luteolin of control and AMF inoculated roots of sorghum accessions.

**Methods S1** Composition of Hoagland solution.

**Methods S2** DNA extraction and real time qPCR analysis.

**Methods S3** Metabolomics analysis.

**Methods S4** Analysis of specialized metabolites.

**Notes S1** Detailed results of sugars, blumenols and flavonoids.

**Table S1** Detailed information of primers used in the qPCR analysis.

**Table S2** List of primary metabolites of control and arbuscular mycorrhizal fungi (AMF)-colonized roots.

**Table S3** List of tentatively identified specialized metabolites of control and arbuscular mycorrhizal fungi (AMF)-colonized roots.

**Table S4** List of tentatively identified blumenol derivatives in control and arbuscular mycorrhizal fungi (AMF)-colonized roots based on tandem mass spectrometry (MS/MS) fragmentation pattern and accurate mass.

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