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2 **Dual RNA-seq reveals large-scale non-conserved genotype x genotype specific genetic**
3 **reprograming and molecular crosstalk in the mycorrhizal symbiosis**

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5 Running title: ***Dual RNA-seq in AM symbiosis***

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16

Abstract

Arbuscular mycorrhizal fungi (AMF) impact plant growth and are a major driver of plant diversity and productivity. We quantified the contribution of intra-specific genetic variability in cassava (*Manihot esculenta*) and *Rhizophagus irregularis* to gene reprogramming in symbioses using dual RNA-sequencing. A large number of cassava genes exhibited altered transcriptional responses to the fungus but transcription of most of these plant genes (72%) responded in a different direction or magnitude depending on the plant genotype. Two AMF isolates displayed large differences in their transcription, but the direction and magnitude of the transcriptional responses for a large number of these genes was also strongly influenced by the genotype of the plant host. This indicates that unlike the highly conserved plant genes necessary for the symbiosis establishment, plant and fungal gene transcriptional responses are not conserved and are greatly influenced by plant and fungal genetic differences, even at the within-species level. The transcriptional variability detected allowed us to identify an extensive gene network showing the interplay in plant-fungal reprogramming in the symbiosis. Key genes illustrated that the two organisms jointly program their cytoskeleton organisation during growth of the fungus inside roots. Our study reveals that plant and fungal genetic variation plays a strong role in shaping the genetic reprograming in response to symbiosis, indicating considerable genotype x genotype interactions in the mycorrhizal symbiosis. Such variation needs to be considered in order to understand the molecular mechanisms between AMF and their plant hosts in natural communities.

Key words: dual RNA-seq, AMF, *Rhizophagus irregularis*, *Manihot esculenta*, cassava, symbiosis, genotype x genotype interaction, mycorrhiza, arbuscular mycorrhizal symbiosis

42 **Introduction**

43 Arbuscular mycorrhizal fungi (AMF) are soil microorganisms that are present in terrestrial
44 ecosystems worldwide [1] and that associate with approximately 74% of land plants [2]. Arbuscular
45 mycorrhizal fungi enhance plant nutrient uptake, plant community diversity and productivity [3],
46 confer plant protection against pathogens and herbivores [4] and protection against abiotic factors
47 such as drought [5] and salt stress [6].

48 In nature, an individual AMF encounters a variety of different species and genotypes of plants. In
49 natural communities, an individual AMF does not appear to show strong preferences for a given
50 plant species or plant genotype [7]. Despite this, plant species respond in different ways to
51 colonization by the same fungus and the plant response spans the range from positive to negative
52 [8]. Moreover, even different sibling fungi of the same AMF parent, which must be genetically very
53 close to each other, can lead to enormous variation in plant growth [9].

54 In order to understand the molecular mechanisms of the mycorrhizal symbiosis, the identification of
55 genes involved in the symbiosis has largely relied on forward genetics [10]. This approach relies on
56 identifying the genetic cause of a clear qualitative phenotype. Researchers have mostly focussed
57 on plant mutants in which impaired fungal development occurs. Most of these studies have been
58 conducted at the very early stages of the association, in many cases for less than 21 days post-
59 inoculation, even though the symbiosis lasts for the lifetime of the plant [11]. This approach has
60 uncovered plant genes that are crucial for the colonization and development of the fungus inside
61 the plant; the so-called “mycorrhizal symbiosis toolkit”. These genes are extremely highly
62 conserved throughout much of the plant kingdom [12]. However, using a forward genetics
63 approach with plant mutants that exhibit impaired fungal development, limits the discovery of
64 genes to those that are essential for the establishment of the symbiosis. The fact that the
65 mycorrhizal toolkit of plant genes is highly conserved among plant species that are capable of
66 forming the mycorrhizal symbiosis is highly consistent with experimental and ecological studies
67 indicating that there is little specificity in the symbiosis; in other words, the genetic programing
68 exists in most terrestrial plants to host mycorrhizal fungi. But the approach used to find these

genes has also lead to the widespread belief that the plant controls the fungus. There may be a bias towards this assumption based on the particular mutant phenotypes that have been chosen for study, i.e. plant mutants that lead to impaired fungal development.

However, ecological studies show that following establishment of the symbiosis, plant species vary enormously in their growth response to a given AMF taxa [3,8,13] or isolate [14-18]. In addition, different genotypes of the same plant species sometimes, but not always, respond differently to inoculation with a given AMF isolate [19-21]. Thus, the beneficial outcome of the association for the plant is not a foregone conclusion just because upregulation of the symbiotic toolkits of genes occurs and the symbiosis becomes physically established. Thus, while we would expect expression of a conserved repertoire of 'mycorrhizal toolkit' genes occurs in all such interactions, we also predict a complex underlying pattern of molecular reprogramming to occur that is plant host genotype and fungal genotype specific.

While significant progress has been made in discovering plant genes that are in involved in the establishment of the symbiosis, identification of important fungal genes involved in these interactions are extremely limited [22-24]. Firstly, this is because stable transformation of AMF has not yet been achieved [25]. Secondly, AMF cannot be cultured without a host. Thirdly, the use of plant mutants that impair fungal development preclude the detection of fungal genes involved in the symbiosis. Finally, because of the lack of knowledge of fungal gene regulation in the symbiosis how plant and fungal genes are co-orchestrated during the symbiosis remains to be elucidated.

Most molecular studies have only evaluated plant transcriptional responses to the fungus by inoculating one plant cultivar with one AMF isolate and comparing the transcriptional response to a mock-inoculated control, without the fungus. Consequently, there is no information on how plant or fungal genetic variation affects plant transcriptional responses to the fungus. Furthermore, there are very few examples where the fungal transcriptional response to different conditions has been measured [26], and to our knowledge, there are no studies that measured the gene transcriptional variation of both partners within the same experimental design. Because of this, it has also not

been possible to study how variation in gene transcription of plant genes is related to variation in gene transcription of the AMF partner.

In this study, we aimed to understand the contribution of intra-specific genetic variation of a host plant and an AMF species to the response of each partner at the gene transcription level. In addition, we aimed to identify plant and fungal molecular interactions that are co-orchestrated during the symbiosis. These aims are ecologically relevant because in nature AMF consistently encounter genetically variable partners, and *vice-versa,* and yet the molecular implications of this for the symbiosis are completely unexplored. Understanding how much the molecular interactions between plant and fungus can be shaped by the plant or fungal genotype has been ignored up to now, although the assumption that plant molecular responses to AMF colonization are so strongly conserved has probably hindered advances in our understanding. Consequently, we established a randomized experimental design comprising five genetically different cultivars of *Manihot esculenta*, each inoculated with two different *Rhizophagus irregularis* isolates and a mock-inoculated control. We measured the plant growth response and AMF colonization in each of the treatments. In addition, we performed a dual RNA-seq experiment, to obtain a genome-wide gene transcription profiling of each plant-AMF combination. This combined dataset allowed us to test quantitatively how plant and fungal gene-transcription are affected by the intra-specific variability of the partner organism. In addition, it allowed us to detect plant and fungal genes that are co-transcribed. Such analyses can only be possible with a dataset in which significant variation in both fungal and plant transcripts is the result of different experimental treatments.

We hypothesized that underneath a common “mycorrhizal toolkit” of conserved genes that determines whether the symbiosis will be established, there is considerable non-conserved transcriptional variation of co-regulated genes in the plant and the fungus that is manifested in associations of varying benefit to the plant. Given that the overriding interest in the symbiosis is due to the potential of the fungus to greatly improve plant growth, the lack of knowledge about gene interactions between the two partners represents a fundamental lacuna in our much-needed understanding of the molecular genetics of this symbiosis.

122 We chose Cassava as the host plant because it is a plant that is known to respond strongly to AMF
123 inoculation in the field [27]. Cassava is considered a vital crop for food security that provides an
124 important source of calories in tropical countries [28]. A good quality cassava genome assembly
125 has recently been published [29]. In addition, cassava can be grown clonally which reduces within-
126 cultivar genetic variation that facilitates the detection of between-cultivar variation. We also focused
127 on *Rhizophagus irregularis* as AMF partner, as it is the main AMF model species with publicly
128 available genome and transcriptome sequences [30-33]. This fungus has been shown to promote
129 the growth of several plants [14], including cassava [34].

130 **Materials & Methods**

131 *Biological material and growth conditions*

132 We designed a two-factor experiment, where we used five cassava (*Manihot esculenta* Crantz)
133 cultivars inoculated independently with two different mycorrhizal fungi or a mock-inoculated control.
134 The five cassava cultivars were CM6438-14, COL2215, BRA337, CM4574-7 and CM523-7. We
135 carried out a greenhouse experiment with a randomized design to avoid block effects
136 (Supplementary note S1).

137 *Trait measurements and statistical analyses*

138 After 18 weeks following inoculation, plant height was measured. We then collected and dried the
139 plants for eight days at 72°C. Dry weight of the shoots, bulking roots and fine roots was measured
140 separately. We also randomly selected some fine roots to measure colonization by AMF
141 (Supplementary note S2). We used a binomial generalized linear model for the analysis of the fine
142 root colonization measurements. For all the other quantitative trait variables, we used a mixed
143 model including block as a random effect and tested the significance of the fixed effect of the
144 cassava cultivar, the AMF treatment and their interaction.

145 *Dual RNA-seq and bioinformatic analysis*

146 We randomly selected several fine root samples from each plant. We extracted RNA, prepared the
147 libraries and sequenced the samples (Supplementary note S3). We then processed the raw reads
148 and separated *in-silico* the cassava and fungal reads (Supplementary figure 1; Supplementary note
149 S4; refer to Supplementary table S1 for the sequenced reads per sample). We then performed a
150 differential expression analysis of each gene (Supplementary note S5) and identified orthologous
151 genes involved in the mycorrhizal symbiosis in *M. esculenta* (Supplementary note S6). The
152 genome assembly and annotation available for cassava (Manihot_esculenta_v6.1) was obtained
153 from the plant genomics resource Phytozome (https://phytozome.jgi.doe.gov/). The *R. irregularis*
154 gene repertoire was predicted (Supplementary note S7, Supplementary data 1). Finally, we

155 performed gene ontology analysis, functional classification and inferred pathway diagrams of the
156 differentially transcribed genes (Supplementary note S8).

157 *Gene co-transcribed networks between cassava and AMF and plant quantitative traits*

158 We used a weighted gene co-expression network analysis (WGCNA) [35] in order to separately
159 build cassava and *R. irregularis* gene modules (clusters of genes displaying similar correlated
160 patterns of transcription). As, we were interested in genes involved in the symbiosis, we used the
161 pool of cassava genes that were significantly differentially expressed between the two AMF
162 treatments and the non-mycorrhizal treatment to build cassava gene modules. On the other hand,
163 because AMF can only be grown in presence of the host plant, we could not select AMF genes that
164 were only involved in the symbiosis. We, therefore, selected all the *R. irregularis* genes in the
165 dataset in order to build *R. irregularis* gene modules. We used the default WGCNA 'step-by-step
166 network construction' analysis to build modules. We first calculated the adjacency between genes
167 and constructed a topological overlap matrix. We then produced a hierarchical clustering tree with
168 the dissimilarity of the topological overlap matrix and we selected the modules by using the
169 dynamic three cut standard method. Finally, we merged similar modules by calculating the module
170 eigengenes, clustering them and assigning a distance threshold. The parameters used were soft-
171 power of 12 and minimum module size of 50 genes. We merged the modules with a distance
172 threshold cut of 0.1.

173 We associated the modules between the two organisms by measuring the Pearson correlation
174 between cassava module eigengenes and *R. irregularis* module eigengenes using the default
175 WGCNA 'relating modules to external information' analysis. We calculated the gene significance
176 (Pearson correlation) for each gene to the corresponding correlated module (e.g. gene1-cassava
177 vs. module1- *R. irregularis* etc.) and we calculated the connectivity (membership) of each gene to
178 its module. We used this information in order to obtain a set of 'key genes' of a module. These are
179 defined as the genes that are the most correlated to modules of the other organism (in the top 10%
180 quantile) and that also displayed a high module membership (>0.8). All the module pairwise

181 comparisons result in a dataset of 'key genes' that are highly connected within each module and
182 highly correlated to modules of the partner organism.

183 We also performed the same analyses as before ('relating modules to external information') to
184 correlate the cassava and *R. irregularis* modules to fungal and plant growth variables. We used the
185 same parameters and threshold cut-off for these comparisons.

186 **Results**

187 *Plant growth and fungal colonization*

188 We observed almost zero colonization levels of the cassava plants in the mock-inoculated
189 treatments, confirming that these treatments were not colonized by AMF (Supplementary figure
190 2a). The cassava cultivars displayed significantly different levels of AMF colonization, but
191 colonization by isolates B1 and DAOM17978 was not statistically different (Supplementary Figure
192 2a; Supplementary Table 2a). We observed an overall effect of the plant cultivar on all the
193 quantitative trait measurements, but we did not observe an overall effect of the fungal identity on
194 the quantitative trait measurements (Supplementary Table 2). Despite this, we observed that the
195 dry weight of cultivar COL2215 was significantly higher when inoculated with isolate B1 compared
196 to the mock control (Supplementary Figure 2b; Supplementary Table 2b).

197 *Activation of plant 'mycorrhizal toolkit' genes in presence of AMF*

198 In order to test the robustness of the plant RNA-seq data, we identified 12 cassava orthologs of
199 genes that are known to be differentially transcribed in response to AMF inoculation in *Medicago*
200 *trunculata* (Supplementary note S6). As expected, this set of genes, which is part of the conserved
201 plant "mycorrhizal toolkit", was clearly differentially transcribed between the mycorrhizal and non-
202 mycorrhizal treatments in all the cultivars (Figure 1; Supplementary Table 3).

203 *Cassava gene transcriptional response to the identity of the fungal partner is plant genotype*
204 *specific*

205 A large number of cassava gene transcripts were recovered from the roots, representing 69% of
206 the total predicted gene number (Figure 2a). Of the total number of transcribed genes, 10193
207 genes (44.6%) were significantly differentially affected by plant genetic variability or the mycorrhizal
208 status of the plants, or both. We did not observe any sequencing bias among the samples, as the
209 number of different cassava gene transcripts did not differ significantly among mycorrhizal
210 treatments, or among cassava cultivars (Supplementary Figure 3a-c), indicating that the dataset
211 was reliable for detecting true transcriptional differences. The transcription of 8559 cassava genes

212 differed significantly among the genetically different cassava cultivars but was not affected by the
213 mycorrhizal symbiosis (Figure 2a; Supplementary Table 4a). Only a small number of cassava
214 genes (450 genes) were influenced by the mycorrhizal treatments in a conserved way, meaning
215 that they were not differentially transcribed among cassava cultivars; with most differences
216 occurring between the mock-inoculated and mycorrhizal plants (Figure 2a; Supplementary Table
217 4b). Only 71 cassava genes were differentially transcribed in plants inoculated with the two
218 different fungal isolates and that also showed consistent transcription patterns among all cassava
219 cultivars (Figure 2a; Supplementary Table 4b). In contrast we observed a strong genotype x
220 genotype interaction on gene transcription as the transcription of a surprisingly large number of
221 cassava genes (1184 genes) was significantly affected by the combination of the genetic identity of
222 both the plant and the mycorrhizal fungus (Figure 2a; Supplementary Table 4c). This means that
223 transcription of those cassava genes were influenced by the presence of the fungus but that the
224 magnitude of this response to the fungus differed quantitatively and in direction among the plant
225 cultivars. From this number, thirty-five cassava genes, displaying large fold changes in transcription
226 between the two fungal treatments, DAOM 197198 and B1, responded in a significantly different
227 way among the cassava cultivars (Figure 2b; Supplementary Table 4d). Many genes involved in
228 the response to biotic and abiotic stresses, categorized as being involved in signalling, secondary
229 metabolites, proteolysis and cell wall constituents were differentially transcribed in a different
230 direction among cultivars inoculated with the different fungal isolates (Supplementary Figure 4a).

231 *Fungal gene transcription is strongly affected by genotypic variation in cassava*

232 The 7840 recovered fungal gene transcripts represented 49% of the predicted gene number
233 (Figure 2a). Very few fungal transcripts were recovered from samples that were mock-inoculated
234 and read number did not influence the number of gene transcripts, showing that there was no bias
235 in the fungal transcriptome data (Supplementary Figure 3a-c). Genetic variation among the
236 cassava cultivars had a strong effect on fungal gene transcription, where 1247 fungal genes were
237 differentially transcribed among cassava cultivars (representing 69% of the differentially
238 transcribed fungal genes; Figure 2a; Supplementary Table 5a). Differential transcription of a
239 smaller number of genes (688 genes) occurred between the two fungal isolates irrespective of the

240 cassava cultivar (Figure 2a; Supplementary Table 5b). We also observed a genotype x genotype
241 interaction as a substantial number of fungal genes (560 genes, representing 31% of the
242 differentially transcribed fungal genes) were differentially transcribed between fungal isolates but in
243 a different direction or magnitude in different cassava cultivars (Figure 2a; Supplementary Table
244 5c), showing lack of conservation in their expression. Of these 560 genes, a high number of fungal
245 genes (269 genes) showed large fold changes in transcription between the two fungi but differed
246 strongly in transcription among cassava cultivars (Figure 2c; Supplementary Table 5d). Genes
247 involved in the auxin pathway, signalling, glutathione-S-transferase, cell wall constituents,
248 proteolysis and MAP kinases and many genes involved in secondary metabolite production were
249 significantly transcribed in different directions in response to cassava genetic variability
250 (Supplementary Figure 4b).

251 *Gene co-transcribed networks as a tool to decipher molecular interactions of the mycorrhizal*
252 *symbiosis*

253 The measurement of plant and fungal gene transcription, and its variability within the same
254 experiment caused by different treatments, allowed us to evaluate co-ordinated molecular
255 interactions between cassava and the fungi by identifying genes of the plant and the fungus that
256 were highly correlated. We used the variance in gene transcription that resulted from the
257 experimental design in order to identify co-transcribed plant and fungal gene clusters. The
258 weighted gene co-expression network approach (WGCNA) allowed us to identify 9 and 20 modules
259 of co-transcribed plant and fungal genes, respectively (Figure 3a, Supplementary Figure 5ab;
260 Supplementary Table 6ab). Within the identified modules, we detected 7 cassava gene modules
261 and 10 fungal gene modules that were correlated to at least one module of the partner species
262 (Figure 3a; Supplementary Figure 5c). To confirm that the modules were biologically meaningful we
263 randomly attributed genes to cassava and fungal modules and attempted to construct a new gene
264 co-transcribed network. None of these modules corresponded to significant gene ontology terms
265 and yielded no network (Supplementary Figure 6). Thus, the observed gene co-transcribed
266 network was unlikely to have occurred by chance. We calculated the gene connectivity within the
267 modules in all genes found in modules that were correlated to a partner module and the gene's

268 correlation to the partner module. This allowed us to detect 'key genes' within a given module (for
269 an example of correlation between 2 modules see Figure 3b). Within all the plant and fungal
270 correlated modules, we found 120 cassava and 317 *R. irregularis* 'key genes' in total that were
271 highly representative of the modules and highly correlated to a counterpart partner module
272 (Supplementary Table 6cd).

273 This approach allowed us to detect several genes with different functions that were correlated with
274 genes within the same organism and the partner organism. For example, we found 10 key genes
275 involved in the fungal secretory pathway that displayed either highly positive or highly negative
276 correlations to other fungal and plant genes. This suggests that the function of this group of genes
277 is heterogeneous, and reveals that approximately half of the genes involved in the secretory
278 pathway are antagonistic (Figure 4; Supplementary Table 6cd). Notably, 8 of these genes were
279 either strongly positively or negatively correlated with genes that are expected to play an important
280 role in the symbiosis such as plant phosphate transporters and sucrose synthases, suggesting an
281 indirect correlation of the secretory pathway in the genetic reprogramming of the symbiosis
282 (Supplementary Table 7). We also found that several key fungal and plant genes in the network
283 represented genes involved in the cytoskeleton and cellular organization. These genes were highly
284 positively correlated between the two organisms (Figure 5; Supplementary Table 6d).

285 *Transcription of plant and fungal genes correlated with fungal colonization*

286 We applied the WGCNA method to detect plant and fungal genes that were correlated with
287 quantitative traits of either fungal or plant growth. We found 3 plant and 3 fungal gene modules that
288 were correlated with quantitative traits. Within these modules, we found that two *R. irregularis*
289 modules and one cassava module correlated with fungal colonization (Figure 6). The fungal
290 module "F_black" and "P_magenta", where correlated with fungal colonization, as well as being
291 highly co-correlated each other (Supplementary Table 6d). Notably, fungal chitin synthase was
292 highly correlated with plant sucrose synthase transcription. In addition, both of these genes were
293 highly correlated with fungal colonization (Figure 6).

294 **Discussion**

295 In this study, we evaluated plant and fungal quantitative growth responses and transcriptional
296 patterns of both partners in response to intraspecific genetic variation. The main finding of this
297 study is that contrary to what is already known about the highly conserved plant molecular
298 responses allowing colonization of roots by mycorrhizal fungi, we found a very large plant genotype
299 x fungal genotype interaction on gene transcription in both organisms, involving an unexpectedly
300 large number of plant and fungal genes. What this means is that genetic reprogramming occurred
301 in cassava roots in response to AMF, but that the magnitude and direction of the transcriptional
302 reprogramming was not conserved, differing greatly among cassava cultivars and fungal
303 genotypes (genotype x genotype interaction). In addition, the genetic variation, used as treatments
304 in this experiment, resulted in considerable quantitative differences in gene transcription levels in
305 the partner organisms that allowed us to detect genes that were highly correlated between the two
306 organisms, as well that were correlated to quantitative growth traits. Such detection of plant-AM
307 fungal gene networks has not been possible in previous studies using simpler experimental
308 designs. The ecological implications of these findings are discussed below.

309 *Conserved and non-conserved molecular reprograming in the mycorrhizal symbiosis and its*
310 *ecological relevance*

311 One of the remarkable features of the last two decades of investigation into plant genes involved in
312 the arbuscular mycorrhizal symbiosis is the fact that a very large number of the plant genes that
313 are involved in the establishment of arbuscular mycorrhizal fungi inside the roots are highly
314 conserved across a large part of the plant kingdom, including basal plant groups (Delaux Ané et
315 al). Coupled with approaches using plant mutants, where disruption of these genes leads to
316 impaired development of the fungus inside the roots, this has lead to the assumption that the
317 molecular basis for the interaction between plants and AM fungi is highly controlled by the plant
318 and also expected to be highly conserved among plants that are capable of forming the
319 mycorrhizal symbiosis. The results of our study clearly showed that a large number of these plant
320 genes were upregulated across all treatments where the fungus was present and irrespective of

321 plant or fungal genetic variation, supporting the hypothesis that fundamental genes involved in the
322 physical establishment of the fungus inside the host root are indeed conserved.

323 Despite this conservation of genes involved in the establishment of the symbiosis, our study shows
324 that following symbiosis establishment, the molecular reprograming that occurs in both the plant
325 and the fungus is highly plant and fungal genotype specific and differentially affects the
326 transcription of a very large number of genes in both organisms. In fact, 72% of the cassava genes
327 that were affected by the mycorrhizal symbiosis were transcribed in a different direction or
328 magnitude among the cassava cultivars and, thus, responded in a non-conserved manner. While
329 previous molecular studies of the symbiosis have shown conservation across plant species,
330 families and orders, we show that the molecular reprogramming during symbiosis is very highly
331 affected by genetic variability, even among closely-related genotypes of one plant species and one
332 fungal species, indicating the high degree of molecular complexity of the interaction following
333 symbiosis establishment and a lack of conservation.

334 In soil, a mycorrhizal fungus repeatedly encounters roots of different plants (different plant species
335 and different genotypes within plant species). *Vice versa*, in soil, a plant encounters different
336 mycorrhizal fungal species and genotypes of a species. Ecological studies show that the outcome
337 of the different combinations of plant and fungal genotypes can lead to very variable outcomes in
338 terms of growth benefits for both individuals and the outcome (at least in terms of growth) is not
339 always positive. In this study, plant and fungal growth varied as a result of the genetically variable
340 treatments and yet upregulation of the conserved repertoire of genes that allowed the
341 establishment of the symbiosis occurred irrespective of whether the establishment was beneficial
342 or not. Based on previously published ecological studies, coupled with the results of this study, we
343 propose that underlying the highly conserved molecular regulation allowing symbiosis
344 establishment, a highly non-conserved genetic reprograming occurs in the plant and the fungus
345 that is highly dependent on the genotype of the partner, even within species. The implications of
346 this are that in order to understand the molecular interactions between the plant and the fungus
347 that determines the fitness benefits afforded by associating with a given partner requires in depth

348 studies that consider genetic variability of both plants and the fungus, even at the level of
349 intraspecific diversity.

350 In this study we observed a non-conserved response of the cassava cultivars to the AMF isolates,
351 particularly in genes involved in plant defence, signalling, cell wall components and proteolysis.
352 This implies that the host plant could elicit a different strategy to the colonization by different AMF
353 genotypes and we hypothesize that depending on the evolutionary history and adaptation of
354 different genotypes, their capacity to perceive and respond to the fungus differs.

355 *Conserved and non-conserved fungal transcriptional responses to cassava and its genetic*
356 *variation*

357 We were able to separate AM fungal gene transcription patterns that were conserved across plant
358 genotypes and those that were non-conserved; varying in direction and magnitude of
359 transcriptional patterns as a result of plant genetic variation. Because almost all published
360 transcriptome studies on the AM symbiosis use an experimental design with one inoculated plant
361 genotype compared to a mock inoculated control, this does not allow the detection of differential
362 AMF gene transcription as the control treatment cannot contain fungal transcripts. Thus, this is the
363 first investigation revealing the conserved *versus* non-conserved genetic reprograming in the
364 fungus during the mycorrhizal symbiosis. The non-conserved fungal genes, among others, were
365 related to the auxin hormone pathway, which is known to be involved in the development of
366 arbuscules [36], glutathione-S-transferases that could be involved in heavy metal binding [37] and
367 MAP Kinases which are known to play a role in the response to biotic and abiotic stresses [38, 39].
368 These results demonstrate that the two AMF strains could have different symbiotic strategies and
369 that these strategies could be modified in presence of a different host plant. Evidence of genotype
370 x genotype interactions on fungal quantitative traits has been already described in AMF [40, 41]. In
371 this study we confirmed, by measuring gene transcription, that genotype x genotype interactions
372 are a common feature that should be addressed to better understand the molecular interactions
373 between both partners.

374 In order to understand the molecular mechanisms of beneficial AMF-plant interactions, a more
375 extensive comparison needs to be made between plants exhibiting benefit from AMF colonization
376 with plants that do not respond, and using different plant models. The identification of a 'beneficial
377 plant mycorrhizal toolkit' would be of great agronomic and ecological interest and would represent
378 a milestone in the applied AMF research.

379 *Gene co-expression networks as a tool to decipher molecular interactions in the mycorrhizal*
380 *symbiosis*

381 The dual RNA-seq approach on AMF colonized plant roots allowed us to identify fungal and plant
382 transcripts that were co-expressed. Dual RNA-seq is an emerging approach that has allowed
383 researchers to decipher interactions between hosts and pathogens [42-45]. The WGCNA approach
384 decreases the complexity of such data sets by clustering gene transcripts and allowing the
385 identification of co-expression patterns between the two organisms, as well as to quantitative traits
386 of the organisms [46]. In this study, we only focussed on the network existing between plant and
387 AM fungal genes, which allowed us to get biological insights into the crosstalk in the mycorrhizal
388 symbiosis. Our study showed that an extensive network of co-expressed gene exists between
389 plant and AM fungal genes. We identified several 'key' plant and fungal genes related to
390 cytoskeleton formation and cell organization that were highly co-expressed within and between
391 both organisms, with a strong positive co-expression of between actin and myosin genes, and a
392 strong negative correlation between actin genes and an actin depolymerisation factor, indicating
393 that plausible biological processes could be detected with this network approach.

394 While the process of arbuscule formation inside the plant cells has been widely described [47],
395 there is little data focusing on the cellular organisation and cytoskeleton changes occurring at later
396 stages of the symbiosis. Cell wall organisation has been studied histologically showing that plant
397 actin filaments and microtubules appear around arbuscular branches, but disappear at arbuscule
398 senescence [48]. In addition, plant microtubules in cells adjacent to fungal structures are also
399 modified, indicating signal exchange between the symbionts prior to fungal penetration [49].
400 However, there is little information about the changes in cellular organisation in the fungal

401 structures, possibly, because more emphasis has been placed on plant structures [50]. Our study
402 shows that there is a strong correlation between genes involved in the cell organisation of the plant
403 and the fungus, which suggest that these processes are probably co-orchestrated.

404 *Correlation of gene-transcription with fungal colonization*

405 We found a strong correlation between fungal colonization of the roots and one plant gene module
406 and two fungal gene modules. Furthermore, both plant and fungal gene modules were highly
407 correlated with each other. Within these modules, notably, we observed a fungal chitin synthase
408 and a plant sucrose synthase that correlated to the fungal colonization. Plant sugar transport, is
409 important for allocation carbohydrates to the fungal symbiont [51]. The co-expressed plant sucrose
410 synthase observed in this study has a conserved domain (TIGR02470), the role of which is to
411 make sucrose available at the cell wall. We hypothesize that the allocation of carbohydrates from
412 the plant to the fungus also has an effect on the fungal colonization phenotype observed in this
413 experiment. We also observed a correlation of the fungal chitin synthase with fungal colonization.
414 Chitin is used by plants as a signal that triggers plant defence [52]. The correlation between the
415 chitin synthase, sucrose synthase and the fungal colonization, suggests that chitin synthase could
416 also have a role in the maintenance of the fungi inside the plant root. We found other 'key genes'
417 that correlated with fungal colonization but their function is less well described. In addition, we
418 classified by functional categories, the 'key genes' found in each module, and we were able to
419 identify strong correlations among and between plant and fungal genes in functional categories
420 (Supplementary table 7). The study in detail of the gene-gene interactions on all the other
421 functional categories is out of the scope of this study. However, the list of genes highlighted in this
422 study could be a starting point to better understand the molecular basis of the interaction between
423 the host plant and the AMF symbiont.

424 *Experimental design in elucidating ecologically relevant molecular interactions between plants and*
425 *AMF*

426 Our study is unique because the experimental treatments of varying plant and fungal genotype
427 allowed us to identify molecular interactions between the plant and the fungus that have not

428 previously been achieved with a conventional mycorrhizal plant *versus* non-mycorrhizal plant
429 comparison. Firstly, this allowed us to investigate the role of genetic variation in the molecular
430 interplay between plants and AM fungi and quantify the conserved *versus* non-conserved
431 component of these molecular interactions; Second, it allowed us to study variation in fungal
432 transcripts which is completely novel; Third, because genetic variation was used as replicated
433 experimental treatments, the resulting variation in plant and fungal gene transcription allowed us to
434 identify a robust co-expression network that exists between the plant and the fungus. None of
435 these results would be identifiable or quantifiable without more complex designs than a simple
436 mycorrhizal versus non-mycorrhizal comparison. While we are fully aware of the enormous
437 advances that have been made in the role of plant genes involved in the establishment of the
438 symbiosis, by the use of plant mutants with impaired fungal development, and that our study of
439 gene transcription cannot experimentally reveal the role of individual genes, our study strongly
440 demonstrates the quantitatively large genotype intraspecific variation in the molecular interactions
441 in the mycorrhizal symbiosis. In order to fully understand the molecular interactions between plants
442 and mycorrhizal fungi that are ecologically relevant and leading to change in plant or fungal fitness,
443 researchers will have to embrace genetic variation in the partners to fully understand these
444 interactions and not rely on simple experimental designs.

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452 the Swiss National Science Foundation (Grant number: 31003A_162549 to IRS). Sequence reads
453 were deposited in the NCBI SRA database (BioProject Accession Number: PRJNA400637).

454

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454

455 **Conflict of Interest** : The authors declare no conflict of interest.

456

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600

601

602 **Figures legends**

603 **Figure 1. Transcription levels of orthologous genes in inoculated and mock-inoculated**
604 **cassava that were previously shown to be involved in the establishment of the arbuscular**
605 **mycorrhizal symbiosis.** Genes were chosen from Hogekamp & Küster, 2013 (Supplementary
606 table 4). We clustered the samples by their similarity in log2-counts per million of each gene and by
607 their similarity in transcription across samples.

608

609 **Figure 2. Variation in cassava and *R. irregularis* gene transcription. (a)** Number of gene
610 transcripts in the different treatments. Cassava genes are reported in green. *R. irregularis* genes
611 are reported in brown. **(b)** Cassava genes with a log2 fold change higher than 2 between plants
612 inoculated with isolates DAOM17978 or B1. **(c)** *R. irregularis* genes with a log2 fold change higher
613 to 4 between isolates DAOM17978 or B1 living with different cassava cultivars**.** Genes (columns)
614 and fold change by cassava cultivar (rows) are clustered by similarity. Gene names can be found in
615 Supplementary table 4d and Supplementary table 5d.

616

617 **Figure 3. Gene co-expression network analysis. (a)** Network of *R. irregularis* and cassava
618 modules. Highly correlated modules (*p-value* <0.001) are linked with a line. Brown and green
619 circles represent *R. irregularis* and plant modules, respectively. **(b)** Example of the identification of
620 key genes between two correlated modules for one plant gene module (module P black) and one
621 fungal gene module (module F magenta). Each dot represents one gene and 'key genes' (the top
622 right of each graph) are the most correlated to modules of the other organism (top 10% quantile)
623 and that also displayed a high module membership (>0.8).

624

625 **Figure 4. Correlogram of *R. irregularis* genes involved in the secretory pathway.** Secretory
626 pathway genes correlated with: **(a)** 50 fungal genes randomly sampled from 317 *R. irregularis* key
627 genes. **(b)** 50 cassava genes randomly sampled from 120 cassava key genes. Each square
628 represents the Pearson correlation coefficient between the genes. Genes in rows and columns
629 were clustered by the similarity of their correlation coefficient. We grouped all the 'key genes' by

630 functional categories and correlated them to all the 'key genes'. All the correlograms made in this
631 study can be found in Supplementary Table 7.
632
633 **Figure 5. Cellular organisation gene transcription in the AM symbiosis. (a)** *R. irregularis* and
634 cassava genes involved in cellular organisation. We show genes involved in cellular organisation
635 that were highly correlated with gene transcription in the partner organism. **(b)** Correlogram of the
636 *R. irregularis* and cassava genes involved in cellular organisation. The information about gene
637 name and function of the 'key genes' is found in Supplementary Table 6.
638
639 **Figure 6. *R. irregularis* and cassava modules correlated with quantitative phenotypic traits.**
640 Correlation matrix between **(a)** the cassava modules, or **(b)** the *R. irregularis* modules (rows) and
641 the quantitative traits (columns). We report the Pearson coefficient and its *p*-value. Highly positive
642 correlations are shown in dark blue and highly negative correlations are shown in dark green. **(c)**
643 Overview of modules and the 'key genes' highly correlated to the quantitative trait fungal
644 colonization. (Gene function and correlation score can be found in Supplementary table 6; for
645 multiple comparison correction threshold refer to Supplementary note S9).
646
647
648

Figure 1

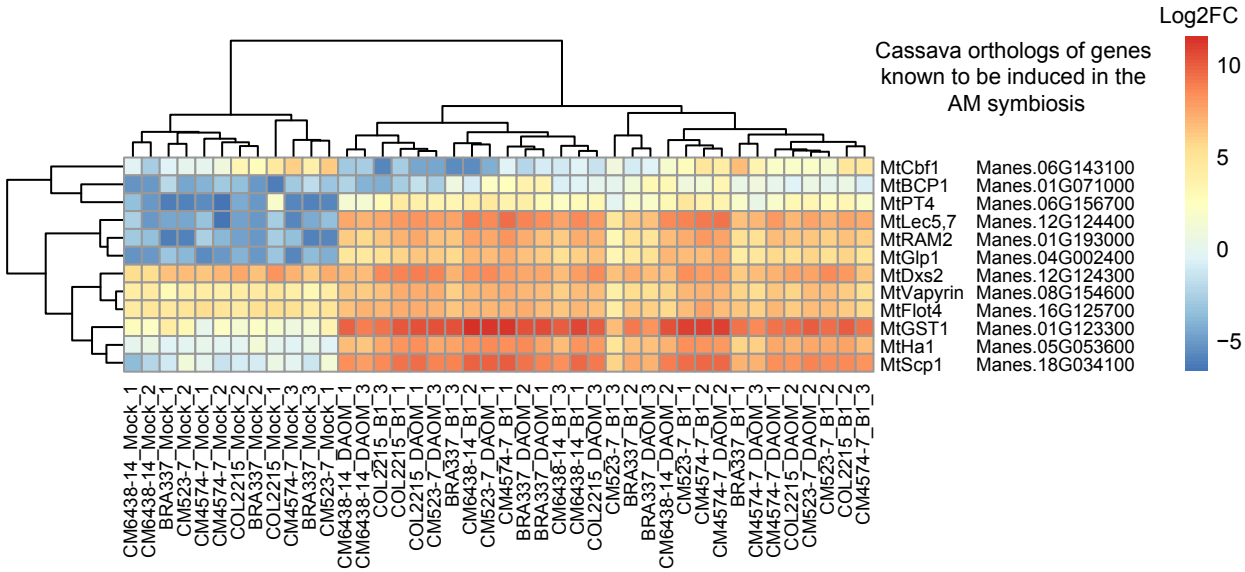
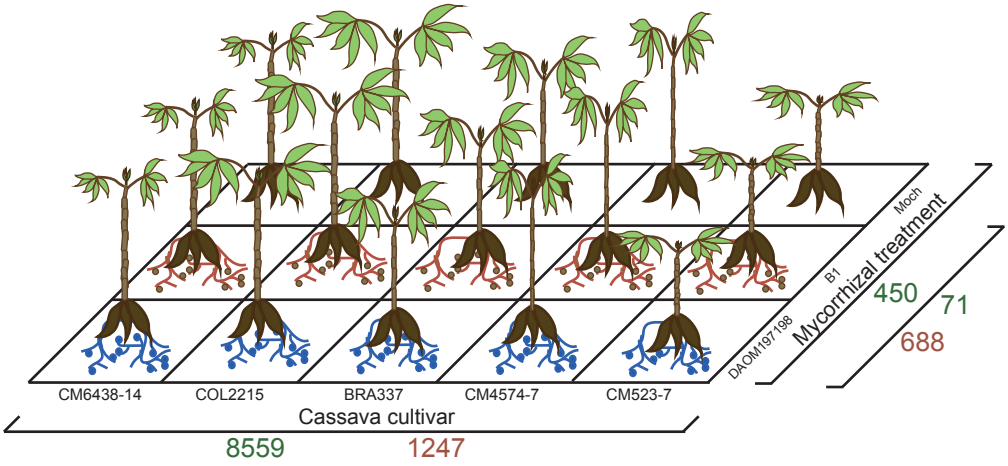

Log2FC
10
5
0
−5
Cassava orthologs of genes known to be induced in the AM symbiosis
MtCbf1 Manes.06G143100
MtBCP1 Manes.01G071000
MtPT4 Manes.06G156700
MtLec5,7 Manes.12G124400
MtRAM2 Manes.01G193000
MtGlp1 Manes.04G002400
MtDxs2 Manes.12G124300
MtVapyrin Manes.08G154600
MtFlot4 Manes.16G125700
MtGST1 Manes.01G123300
MtHa1 Manes.05G053600
MtScp1 Manes.18G034100
CM6438-14_Mock_1
CM6438-14_Mock_2
BRA337_Mock_1
CM523-7_Mock_2
CM4574-7_Mock_1
CM4574-7_Mock_2
COL2215_Mock_2
BRA337_Mock_2
COL2215_Mock_1
CM4574-7_Mock_3
BRA337_Mock_3
CM523-7_Mock_1
CM6438-14_DAOM_1
CM6438-14_DAOM_3
COL2215_B1_3
COL2215_B1_1
COL2215_DAOM_1
CM523-7_DAOM_3
BRA337_B1_3
CM6438-14_B1_2
CM523-7_DAOM_1
CM4574-7_B1_1
BRA337_DAOM_2
BRA337_DAOM_1
CM6438-14_B1_3
CM6438-14_B1_1
COL2215_DAOM_3
CM523-7_B1_3
BRA337_B1_2
BRA337_DAOM_3
CM6438-14_DAOM_2
CM523-7_B1_1
CM4574-7_B1_2
CM4574-7_DAOM_2
BRA337_B1_1
CM4574-7_DAOM_3
CM4574-7_DAOM_1
COL2215_DAOM_2
CM523-7_DAOM_2
CM523-7_B1_2
COL2215_B1_2
CM4574-7_B1_3

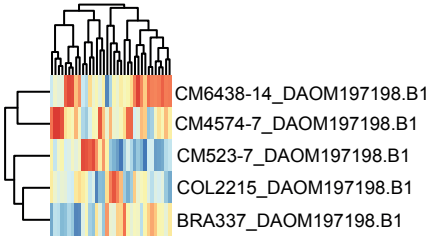
Figure 2

a)



Total predicted genes	33030 15941
Total sequenced genes	22812 7840
Genes unaffected by the treatments	12619 5345
Genes affected by interaction between plant & fungal genotype	1184 560

b)



c)

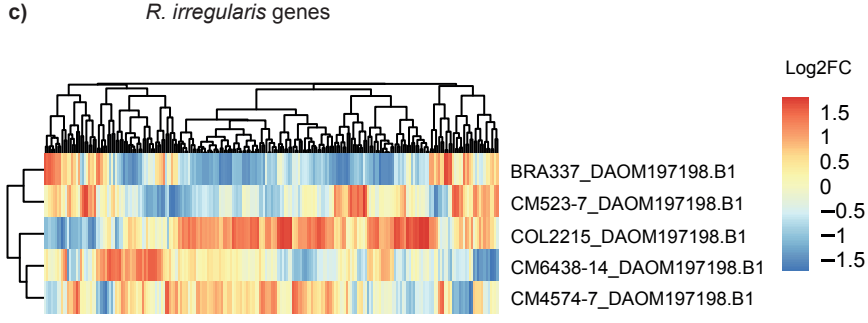
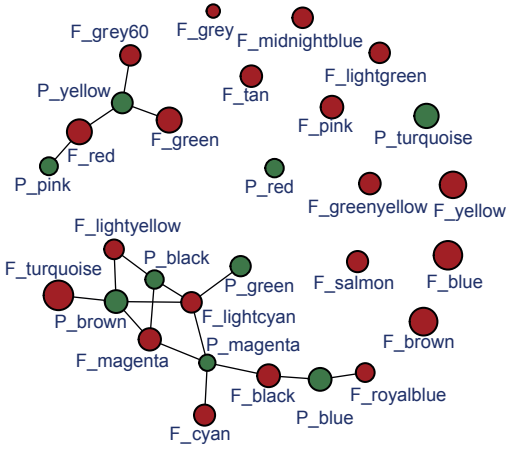


Figure 3

a)


F_grey60
F_grey
F_midnightblue
F_lightgreen
P_yellow
F_tan
F_pink
P_turquoise
F_green
F_red
P_pink
P_red
F_greenyellow
F_yellow
F_lightyellow
P_black
F_turquoise
P_green
F_salmon
F_blue
P_brown
F_lightcyan
P_magenta
F_brown
F_magenta
F_black
F_royalblue
P_blue
F_cyan

b)

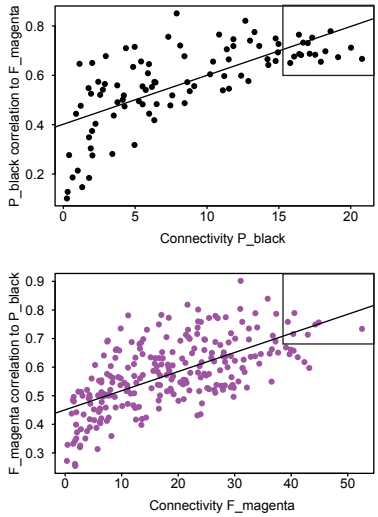

P_black correlation to F_magenta
0.2
0.4
0.6
0.8
0
5
10
15
20
Connectivity P_black
F_magenta correlation to P_black
0.3
0.4
0.5
0.6
0.7
0.8
0.9
0
10
20
30
40
50
Connectivity F_magenta

Figure 4

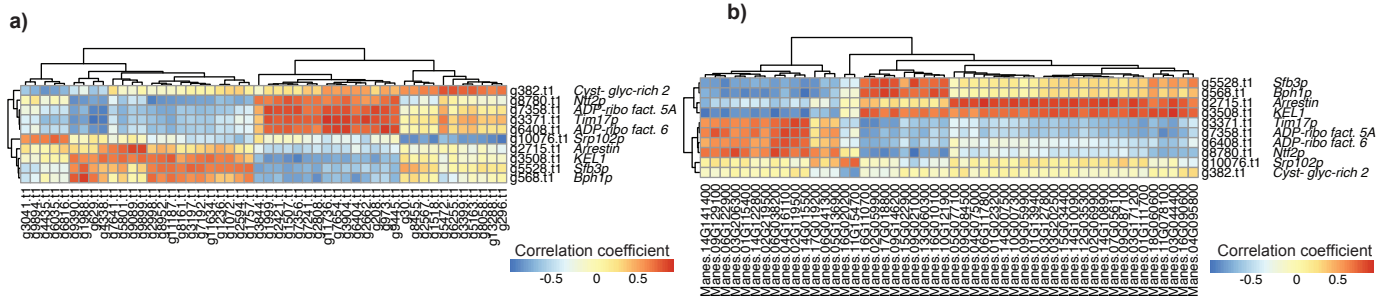
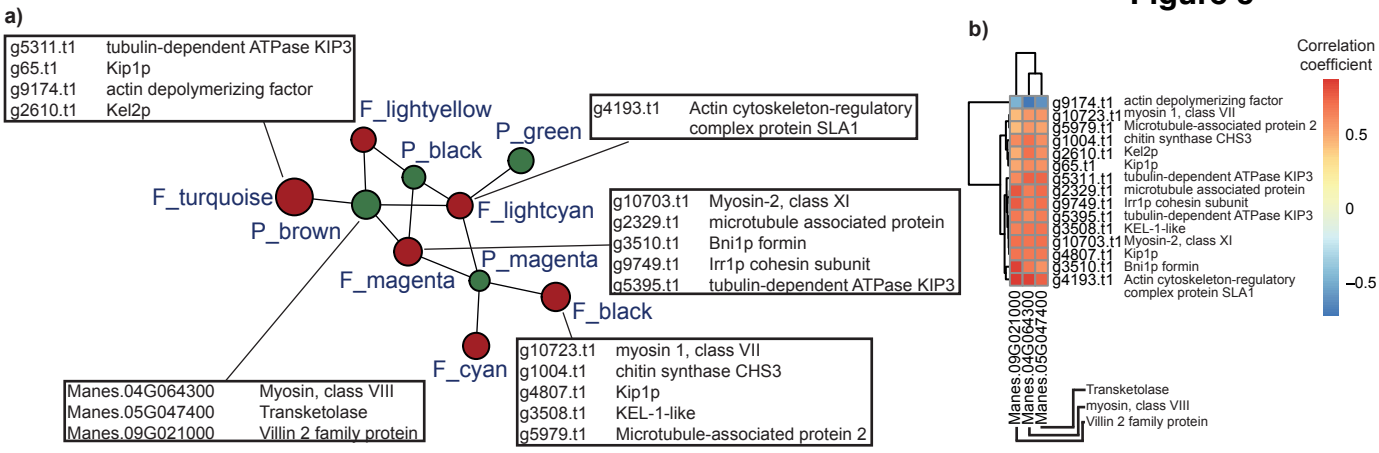

a)
g382.t1 Cyst- glyc-rich 2
g8780.t1 Ntf2p
g7358.t1 ADP-ribo fact. 5A
g3371.t1 Ym17p
g6408.t1 ADP-ribo fact. 6
g10076.t1 Srp102p
g2715.t1 Arrestin
g3508.t1 KEL1
g5528.t1 Sfb3p
g568.t1 Bpn1p
Correlation coefficient
-0.5
0
0.5
b)
g5528.t1 Sfb3p
g568.t1 Bpn1p
g2715.t1 Arrestin
g3508.t1 KEL1
g3371.t1 Ym17p
g7358.t1 ADP-ribo fact. 5A
g6408.t1 ADP-ribo fact. 6
g8780.t1 Ntf2p
g10076.t1 Srp102p
g382.t1 Cyst- glyc-rich 2
Correlation coefficient
-0.5
0
0.5

Figure 5


a)
g5311.t1 tubulin-dependent ATPase KIP3
g65.t1 Kip1p
g9174.t1 actin depolymerizing factor
g2610.t1 Kel2p
F_lightyellow
P_green
P_black
g4193.t1 Actin cytoskeleton-regulatory complex protein SLA1
F_turquoise
P_brown
F_lightcyan
g10703.t1 Myosin-2, class XI
g2329.t1 microtubule associated protein
g3510.t1 Bni1p formin
g9749.t1 Irr1p cohesin subunit
g5395.t1 tubulin-dependent ATPase KIP3
P_magenta
F_magenta
F_black
F_cyan
g10723.t1 myosin 1, class VII
g1004.t1 chitin synthase CHS3
g4807.t1 Kip1p
g3508.t1 KEL-1-like
g5979.t1 Microtubule-associated protein 2
Manes.04G064300 Myosin, class VIII
Manes.05G047400 Transketolase
Manes.09G021000 Villin 2 family protein
b)
Correlation coefficient
0.5
0
−0.5
g9174.t1 actin depolymerizing factor
g10723.t1 myosin 1, class VII
g5979.t1 Microtubule-associated protein 2
g1004.t1 chitin synthase CHS3
g2610.t1 Kel2p
g65.t1 Kip1p
g5311.t1 tubulin-dependent ATPase KIP3
g2329.t1 microtubule associated protein
g9749.t1 Irr1p cohesin subunit
g5395.t1 tubulin-dependent ATPase KIP3
g3508.t1 KEL-1-like
g10703.t1 Myosin-2, class XI
g4807.t1 Kip1p
g3510.t1 Bni1p formin
g4193.t1 Actin cytoskeleton-regulatory complex protein SLA1
Manes.09G021000
Manes.04G064300
Manes.05G047400
Transketolase
myosin, class VIII
Villin 2 family protein

a)

Fungal module − Phenotype relationships

	Fungal colonization	Aboveground dry weht	Fine roots dry weight	Tuberized roots dry weight	Total dry weight	Belowground dry weight	Height
F_blue	−0.089 (0.6)	−0.29 (0.1)	−0.087 (0.6)	−0.042 (0.8)	−0.096 (0.6)	−0.048 (0.8)	−0.17 (0.4)
F_lightcyan	0.21 (0.3)	−0.0021 (1)	0.28 (0.1)	−0.17 (0.4)	−0.15 (0.4)	−0.15 (0.4)	0.13 (0.5)
F_lightgreen	−0.31 (0.09)	−0.42 (0.02)	−0.026 (0.9)	0.0041 (1)	−0.068 (0.7)	0.0024 (1)	−0.21 (0.3)
F_grey60	−0.14 (0.5)	−0.11 (0.6)	−0.036 (0.9)	0.42 (0.02)	0.4 (0.03)	0.42 (0.02)	−0.33 (0.07)
F_pink	−0.27 (0.2)	−0.22 (0.2)	0.063 (0.7)	0.15 (0.4)	0.12 (0.5)	0.16 (0.4)	−0.14 (0.5)
F_yellow	−0.23 (0.2)	0.034 (0.9)	0.14 (0.5)	0.2 (0.3)	0.21 (0.3)	0.21 (0.3)	−0.027 (0.9)
F_midnightblue	−0.28 (0.1)	0.28 (0.1)	0.17 (0.4)	−0.41 (0.03)	−0.34 (0.06)	−0.39 (0.03)	0.41 (0.03)
F_black	−0.54 (0.002)	−0.079 (0.7)	−0.26 (0.2)	−0.27 (0.2)	−0.3 (0.1)	−0.29 (0.1)	0.014 (0.9)
F_royalblue	−0.59 (0.0006)	−0.14 (0.5)	−0.14 (0.5)	−0.18 (0.4)	−0.21 (0.3)	−0.19 (0.3)	−0.00034 (1)
F_tan	−0.45 (0.01)	−0.088 (0.6)	0.048 (0.8)	−0.0098 (1)	−0.021 (0.9)	−0.0065 (1)	0.0046 (1)
F_turquoise	0.37 (0.05)	0.074 (0.7)	0.17 (0.4)	−0.2 (0.3)	−0.18 (0.3)	−0.19 (0.3)	0.23 (0.2)
F_brown	0.43 (0.02)	0.098 (0.6)	0.13 (0.5)	0.14 (0.5)	0.16 (0.4)	0.15 (0.4)	0.083 (0.7)
F_green	0.29 (0.1)	0.12 (0.5)	0.07 (0.7)	−0.062 (0.7)	−0.036 (0.8)	−0.058 (0.8)	0.19 (0.3)
F_greenyellow	−0.3 (0.1)	0.025 (0.9)	−0.12 (0.5)	−0.33 (0.07)	−0.33 (0.07)	−0.34 (0.06)	0.22 (0.2)
F_cyan	−0.16 (0.4)	0.039 (0.8)	−0.21 (0.3)	−0.33 (0.07)	−0.34 (0.07)	−0.35 (0.06)	0.16 (0.4)
F_magenta	−0.15 (0.4)	0.032 (0.9)	−0.21 (0.3)	0.15 (0.4)	0.14 (0.5)	0.13 (0.5)	−0.053 (0.8)
F_red	0.13 (0.5)	0.084 (0.7)	−0.08 (0.7)	0.027 (0.9)	0.035 (0.9)	0.021 (0.9)	0.098 (0.6)
F_lightyellow	−0.18 (0.4)	−0.04 (0.8)	−0.26 (0.2)	0.47 (0.009)	0.44 (0.02)	0.45 (0.01)	−0.32 (0.09)
F_salmon	0.34 (0.07)	0.2 (0.3)	0.22 (0.2)	−0.037 (0.8)	0.012 (0.9)	−0.022 (0.9)	0.12 (0.5)
F_grey	0.026 (0.9)	0.088 (0.6)	−0.031 (0.9)	−0.11 (0.6)	−0.098 (0.6)	−0.11 (0.5)	−0.084 (0.7)

b)

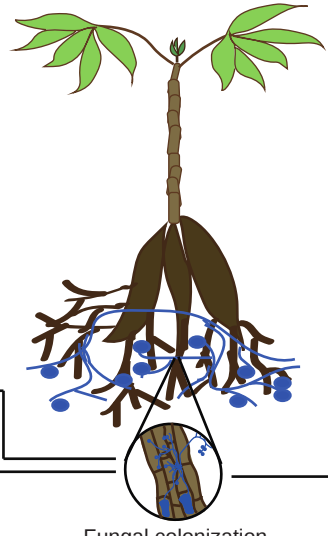
Plant module − Phenotype relationships

	Fungal colonization	Aboveground dry weht	Fine roots dry weight	Tuberized roots dry weight	Total dry weight	Belowground dry weight	Height
P_black	−0.22 (0.2)	−0.17 (0.4)	−0.29 (0.1)	0.3 (0.1)	0.25 (0.2)	0.29 (0.1)	−0.32 (0.08)
P_turquoise	−0.28 (0.1)	−0.35 (0.05)	−0.11 (0.6)	−0.046 (0.8)	−0.11 (0.6)	−0.053 (0.8)	−0.32 (0.08)
P_blue	−0.44 (0.01)	−0.14 (0.5)	−0.28 (0.1)	−0.091 (0.6)	−0.13 (0.5)	−0.11 (0.6)	−0.24 (0.2)
P_magenta	−0.49 (0.006)	0.03 (0.9)	−0.3 (0.1)	−0.094 (0.6)	−0.11 (0.6)	−0.11 (0.5)	−0.052 (0.8)
P_red	0.076 (0.7)	−0.029 (0.9)	−0.14 (0.5)	−0.084 (0.7)	−0.097 (0.6)	−0.094 (0.6)	−0.14 (0.5)
P_yellow	0.1 (0.6)	−0.3 (0.1)	−0.15 (0.4)	0.076 (0.7)	0.014 (0.9)	0.065 (0.7)	−0.5 (0.005)
P_brown	0.43 (0.02)	−0.1 (0.6)	0.29 (0.1)	−0.086 (0.7)	−0.083 (0.7)	−0.066 (0.7)	−0.051 (0.8)
P_green	0.19 (0.3)	0.065 (0.7)	0.56 (0.001)	−0.054 (0.8)	−0.0051 (1)	−0.016 (0.9)	0.35 (0.06)
P_pink	−0.019 (0.9)	0.1 (0.6)	0.4 (0.03)	−0.14 (0.5)	−0.096 (0.6)	−0.11 (0.5)	0.35 (0.06)

c)

R. irregularis key genes

Cassava key genes



Module F_black

g10683.t1 chitin synthase CHS3
g10723.t1 myosin 1
g12269.t1 hypothetical protein RirG_043580
g12890.t1 Phospholipase A1-II 1
g13622.t1 sterol 14-demethylase
g1385.t1 putative serine hydrolase C5E4.05c
g1651.t1 Acyl-coenzyme A thioesterase 12
g2124.t1 hypothetical protein RirG_032760
g2715.t1 Arrestin domain-containing protein C584.15c
g2722.t1 Kch1p
g2937.t1 medium-chain fatty acid-CoA ligase FAA2
g406.t1 Suppressor protein of bem1/bed5 double mutants
g4807.t1 Kip1p
g4952.t1 Ca(2+)/Mn(2+)-transporting P-type ATPase PMR1
g5145.t1 StAR-related lipid transfer protein 5
g759.t1 hypothetical protein GLOINDRAFT_336497

Module F_royalblue

g10583.t1 NA
g10838.t1 Molybdenum cofactor sulfurase
g197.t1 hypothetical protein GLOINDRAFT_335278
g3691.t1 Protein CIP2A
g4948.t1 Transcription factor HIVEP3
g4959.t1 Monoterpene epsilon-lactone hydrolase
g6864.t1 MFS general substrate transporter
g856.t1 hypothetical protein RirG_239000

Module P_magenta

Manes.03G044400 sucrose synthase
Manes.03G103200 Malate dehydrogenase
Manes.07G087500 mevalonate diphosphate decarboxylase
Manes.17G040300 Beta-galactosidase 8

Figure 6