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Research

The mycorrhizal root-shoot axis elicits *Coffea arabica* growth under low phosphate conditions

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Summary

- Coffee is one of the most traded commodities world-wide. As with 70% of land plants, coffee is associated with arbuscular mycorrhizal (AM) fungi, but the molecular bases of this interaction are unknown.
- We studied the mycorrhizal phenotype of two commercially important *Coffea arabica* cultivars ('Typica National' and 'Catimor Amarillo'), upon *Funneliformis mosseae* colonisation grown under phosphorus limitation, using an integrated functional approach based on multi-omics, physiology and biochemistry.
- The two cultivars revealed a strong biomass increase upon mycorrhization, even at low level of fungal colonisation, improving photosynthetic efficiency and plant nutrition. The more important iconic markers of AM symbiosis were activated: We detected two gene copies of AM-inducible phosphate (Pt4), ammonium (AM2) and nitrate (NPF4.5) transporters, which were identified as belonging to the *C. arabica* parental species (*C. canephora* and *C. eugenioides*) with both copies being upregulated. Transcriptomics data were confirmed by ions and metabolomics analyses, which highlighted an increased amount of glucose, fructose and flavonoid glycosides.
- In conclusion, both coffee cultivars revealed a high responsiveness to the AM fungus along their root-shoot axis, showing a clear-cut re-organisation of the major metabolic pathways, which involve nutrient acquisition, carbon fixation, and primary and secondary metabolism.

Introduction

Coffee is one of the most important traded agricultural commodities world-wide, and five countries (Brazil, Vietnam, Colombia, Indonesia and Ethiopia) account for 75% of the world's total coffee production. The coffee sector is vulnerable to fluctuations caused by low productivity levels, climate change effects and damages caused by pests and diseases (e.g. Coffee Berry Borer, Halo Blight, Berry Disease and Wilt Disease) across the globe (Adhikari *et al.*, 2020). The COVID pandemic has unexpectedly raised coffee consumption, which probably mirrors a substantial increase in at-home use, leading to the estimation that the global coffee market will grow at an overall annual growth rate of 4.28% during the 2022–2027 period (Mordor Intelligence, 2018). The forecast of an increase in consumption in the context of global climate changes opens the questions as to how coffee cultivation can satisfy the market demand, while simultaneously answering the request for greater sustainability of cultivation.

Like 70% of land plants living in natural environments (Tedersoo *et al.*, 2020), coffee plants host arbuscular mycorrhizal (AM) fungi in their roots. These soil fungi are obligate symbiotic microbes which develop extraradical hyphae capable of harvesting mineral nutrients, such as phosphorus (Pi) and nitrogen (N), from the soil and delivering them to the host during their intraradical phase when highly branched structures (the arbuscules) are produced inside the root cortical cells (Genre *et al.*, 2020). This process improves the host mineral nutrition and increases the level of plant innate immunity to biotic stresses (Jung *et al.*, 2012; Fiorilli *et al.*, 2018; Pozo de la Hoz *et al.*, 2021). In the meantime, the plants support the fungus's nutritional requests providing reduced carbon in the form of sugars and lipids (Jiang *et al.*, 2017; Luginbuehl *et al.*, 2017).

Many studies have illustrated that in different continents, including South American coffee plantations, natural coffee forest in South-West Ethiopia (Muleta *et al.*, 2007), which is the region of origin of *Coffea arabica* L. (Montagnon *et al.*, 2022), as well as in managed coffee plantations (Muleta *et al.*, 2007),

roots of adult plants are colonised by different AM fungi (Arias *et al.*, 2012). These investigations demonstrate that AM fungal diversity changes depending on the soil features and the management practices (Andrade *et al.*, 2009). According to Júnior *et al.* (2019), agroecological practices seem to increase AM fungal biodiversity in Brazilian coffee plantations. In Peru plantations of *C. arabica*, the phylogenetic diversity of AM fungal communities increases with the plant age (Aguila *et al.*, 2022). On the contrary, it has also been established that *in vitro* propagated coffee seedlings are highly dependent on mycorrhizal fungi (Vaast *et al.*, 1997). The beneficial impact of AM fungi on plant development and yield productivity was followed by Siqueira *et al.* (1998) in a long-term experiment where coffee plants were cultivated in nutrient-poor soil, at different phosphate rates and seven AM fungal treatments. The impact of the fungal inoculation treatment was relevant only in the first bean harvest, even if a positive impact was observed over 6 yr. Fonseca *et al.* (2019) evaluated the effect of *Rhizophagus clarus* inoculation over the initial development and nutritional response of six genotypes of *Coffea arabica* revealing that the inoculation benefits varied according to the genotypes, but led to higher shoot and root dry mass. However, the molecular basis of such phenotypes is so far unknown: while omics techniques allowed deciphering how crop plants (i.e. wheat, maize, tomato and rice) reprogram their metabolism in the presence of AM fungi (Gerlach *et al.*, 2015; Chialva *et al.*, 2018; Fiorilli *et al.*, 2018; Vannini *et al.*, 2021; Fujita *et al.*, 2022; Hsieh *et al.*, 2022), with the exception of grapevine (Balestrini *et al.*, 2017; Goddard *et al.*, 2021), such approaches have rarely been applied to perennial crops. Our question was whether these changes, which in annual crops involve the so-called root-shoot axis and impact plant nutrition, development and immunity (Chialva *et al.*, 2022; Custódio *et al.*, 2022), are also observable in plants with diverse developmental traits and ecological requests. Coffee plants are tropical evergreen shrubs, traditionally grown high in the mountains in the underwood. Due to the relevance of coffee as one of the most marketed products and as a source of direct/indirect income for many countries, we aimed (1) to validate the mycorrhizal phenotype of two commercially important *C. arabica* cultivars, 'Typica National' and 'Catimor Amarillo' which have not been analysed previously, (2) to decipher their responses to an AM fungus by using an integrated functional approach based on 'omics', physiology and biochemistry and (3) to guide strategies to develop more sustainable coffee plants production in nurseries. 'Typica National' is the most renowned variety within the Bourbon-Typica lineage. It probably originated in Southwestern Ethiopia, migrated towards Yemen, India and Java, then arrived at the Botanical Gardens of Amsterdam in the first years of the 18th century from there moved towards South America (Ferreira *et al.*, 2019). Catimor has a much more recent history (Tummakate, 1984): it was selected in Portugal in 1959 by scientists searching for high yields, high disease resistance and small-size traits. This variety is a hybrid of the 'Timor Hybrid' (*C. arabica* × *C. canephora*, resistant to coffee leaf rust due to its *C. canephora* background) and 'Caturra', which has genetic roots in the Arabica Bourbon variety.

Materials and Methods

Plant and fungal material, growth conditions and sampling

Coffea arabica L. seeds of 'Typica National' and 'Catimor Amarillo' varieties kindly provided by Luigi Lavazza S.p.A. were prehydrated for 4 d in 50 ml falcon tubes filled with warm tap water and then sown in moist sterilised quartz sand (180°C, 3 h). After 60 d, the obtained seedlings were transferred into square plastic pots (10 × 10 × 12 cm, 0.90 l volume) filled with sterile quartz sand (nonmycorrhizal control, NM) or with a commercial monospecific *Funnelliformis mosseae* inoculum (MycAgro Lab, Dijon, France) 30% diluted (v/v) in sterile quartz sand (mycorrhizal plants, MYC). This AMF species was chosen given it is a common model in mycorrhiza research and has been shown in Brazil, Mexico and India to colonise coffee roots under field conditions (Cogo *et al.*, 2017). The two coffee varieties were grown over three successive years (Trials 1–3, 2020–2022), either with or without the AMF inoculation (MYC and NM). For the first trial, eight replicates of each variety/condition were evaluated. For the subsequent two trials these numbers were lowered to six replicates, yielding in a total of 74 plants examined throughout the entire experiment. The plants were grown at the Department of Life Sciences and Systems Biology (University of Torino, Italy) under controlled conditions at 23°C : 21°C, day : night temperature with a photoperiod of 16 h : 8 h, light : dark at 150 PFFD light intensity, randomising plants within the growth room. Pots were watered with a modified Long-Ashton nutrients solution (Hewitt, 1966) at 7.5 µM Pi once a week and with tap water as needed to maintain the substrate moist.

Following a preliminary sampling 2 months after inoculation, plants were sampled after 5 months, roots were washed under tap water and biometric values measured, including the number of internodes and leaves, the stem height, the root and shoot fresh weights (RFW and SFW, respectively) and the root/shoot dry weights (RDW and SDW, respectively). Additionally, the SPAD index was measured after 2 months and at sampling using a SPAD-502 Chlorophyll Meter (Konica-Minolta Inc., Chiyoda, Tokyo, Japan) on the first fully expanded opposite leaves (the second or third internode from the apical meristem) averaging the measure of eight randomly selected spots (four per each leaf) for each plant. Subsamples of the root apparatus and of leaf material were then immediately frozen in liquid nitrogen and stored at −80°C until further analyses.

Mycorrhizal colonisation

Arbuscular mycorrhizal colonisation was morphologically quantified in Trials 1 and 3. Immediately after sampling, a representative portion of the root apparatus was used to analyse the mycorrhizal phenotype (100 cm of root for each plant, five different plants for each condition). Roots were stained in methyl blue (0.1% w/v in lactic acid) for 12 h at room temperature, washed in lactic acid 80% (v/v), and their mycorrhizal colonisation intensity estimated (Supporting Information Methods S1; Trouvelot *et al.*, 1986).

RNA sequencing and bioinformatics

Both root and leaf material were used for molecular analysis. First, a genome-wide transcriptome study (mRNA-Seq) was performed on 5-month-old *C. arabica* plants (cv 'Typica National') from Trial 1 under MYC and NM conditions using NovaSeq6000 platform (Illumina, San Diego, CA, USA; 2 × 150 paired-end, 9 Gb per sample). Libraries were mapped on the *C. arabica* reference transcriptome (cv 'Caturra-red'; Zimin *et al.*, 2018) using SALMON v.1.5.1 software (Patro *et al.*, 2017) in selective alignment mode, using the whole genome sequence as 'decoy' (Table S1). Differentially expressed genes (DEGs) were called at the isoform level using DESEQ2 (Love *et al.*, 2014) at FDR < 0.05 and |FC| ≥ 2. Since no functional annotation was available, Gene Ontologies (GOs) and KEGG orthologs were inferred on the protein-coding genes using INTERPROSCAN (Jones *et al.*, 2014) and the KEGG Automatic Annotation Server (KAAS), respectively, and categories enriched among DEGs using GOSEQ (Young *et al.*, 2010). The expression of selected genes (Table S2) was further tested using real-time PCR on an independent experiment (T2).

Additional details on RNA extraction, sequencing, bioinformatics and real-time PCR assays are provided in Methods S1.

Metabolomics and biochemical assays

Metabolomics analyses were performed integrating GC-TOF-MS (primary metabolism) and LC-ESI-MS (secondary metabolism) on both roots and leaf materials from Trial 2 ('Typica National' genotype). The content of major cations and anions was measured using capillary electrophoresis on water extracts obtained from the second/third leaf from the apical meristem of the same plants used for metabolomics (Trial 2).

Photosynthetic pigments (chlorophylls and carotenoids), total phenols and lignin concentration were measured on leaf, stem and root extracts of both genotypes obtained in Trial 2 using spectrophotometric assays. Detailed methods are provided in Methods S1.

Physiological measurements

Gas exchange and pulse-amplitude modulation (PAM) fluorometric analyses on single leaves were performed with a portable GFS-3000 (Walz, Germany) Infra-Red Gas Analyzer (IRGA) on 90 d-old plants from Trial 3. For each condition, five to six plants were measured. Detailed procedures, environmental conditions and measured parameters are detailed in Methods S1.

Statistical analyses

All statistical tests were performed in the R programming environment v.4.2.2 (R Core Team, 2022). Data were checked for normality and homoscedasticity using Shapiro–Wilk (Shapiro & Wilk, 1965) and Levene's test (Levene, 1960), respectively ($P < 0.05$), using 'shapiro.test' and 'leveneTest' functions from the STATS and CAR v.3.1-1 (Fox & Weisberg, 2019) packages. If data followed a normal distribution and variances were homogeneous, the Student's *t*-test ($P < 0.05$) was applied to compare MYC vs NM condition using 't.test' function. If data resulted as non-normal, differences between conditions were tested using the Kruskal–Wallis test ($P < 0.05$; Kruskal, 1952) using 'kruskal.test' function.

Heatmaps were plotted using COMPLEXHEATMAP package v.2.12.1 (Gu, 2022) and principal component analysis (PCA) on ions and metabolomics data obtained running the PRCOMP R function on log-transformed abundances. For LC–MS data, zero-variance variables were removed to allow PCA analysis. All the graphical visualisations were performed using the GGPLOT2 v.3.4.0 R package (Wickham, 2016) with extended functionalities from the LEMON v.0.4.5 (Edwards, 2022) and GGPUBR v.0.5.0 (Kassambara, 2022) libraries.

Results

Mycorrhizal phenotype

Since two seedlings from the Trial 1 revealed only traces of root mycorrhization after 2-month growth, the remaining six were measured and sampled 5 months after the fungal inoculation. Both coffee varieties, grown under controlled low Pi conditions, revealed a strong mycorrhizal growth response upon *Funneliformis mosseae* inoculation (Figs 1, S1). Mycorrhizal (MYC) plants of both varieties exhibited higher biomass compared with non-mycorrhizal controls (NM) with high consistency across the three independent experimental trials performed. The impact at the systemic level was similar for both varieties with more marked differences between MYC and NM conditions in 'Catimor': MYC plants showed increased stem height and an overall fresh biomass accumulation (Fig. 1a). These results were further confirmed by the significant increase in the root and shoot dry weight upon mycorrhizal inoculation in both varieties (Trial 3) and of the internodes and leaves number in the 'Catimor' genotype only (Trial 2, Fig. S2). Notably, MYC plants appeared greener than NM ones (Fig. 1b,c). To validate this visual observation, the SPAD index, which correlates with the relative chlorophyll content and mirrors the total plant N content, was measured at 60 and 150 d from the inoculation (Fig. 1a). MYC plants had consistently higher values than NM plants suggesting that symbiosis led to an improved plant nutritional status. To confirm SPAD index measurements, photosynthetic pigments were quantified spectrophotometrically. In 'Catimor Amarillo', total chlorophyll, chlorophyll a and carotenoid content were higher in MYC plants, while in 'Typica National', differences were less pronounced, although a trend was still apparent (Fig. 1d).

Notwithstanding the strong growth effect (Fig. 1), the quantification of the mycorrhization revealed a relatively low colonisation frequency (F, mean = 27.56%) and intensity (M, mean = 1.12%; Fig. 2a). The 'Typica' variety showed a higher M, F and overall arbuscule abundance (A) compared with 'Catimor'. However, in both genotypes, arbuscules were normally developed in mycorrhizal root segments with an arbuscule

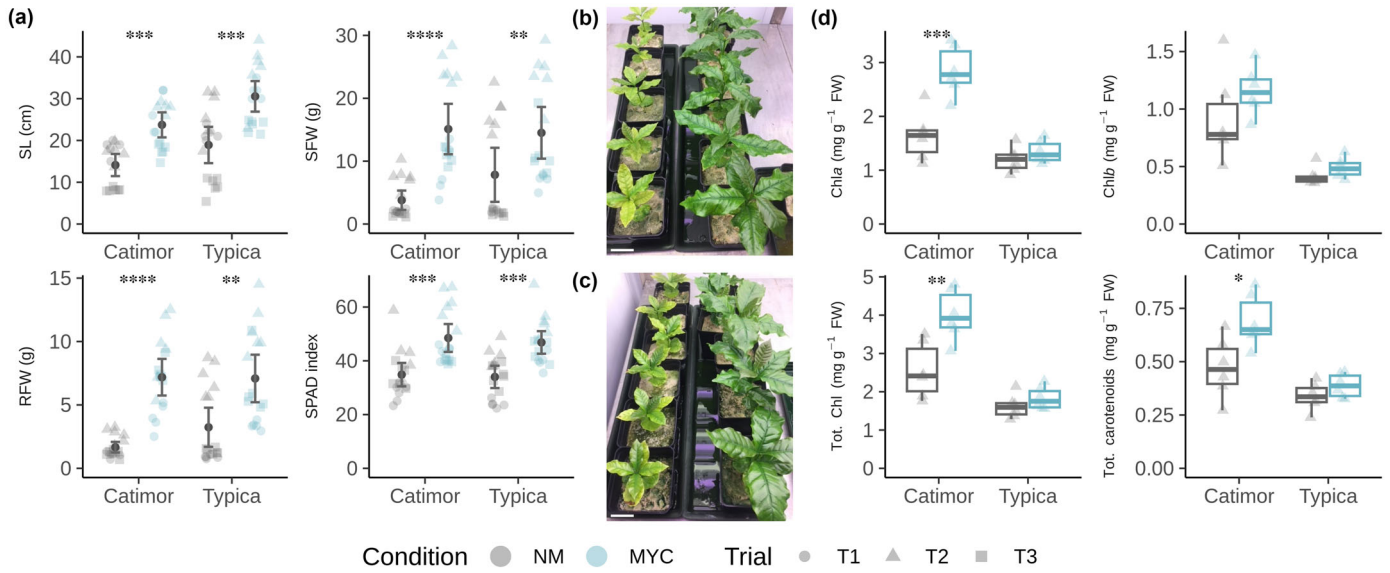

Fig. 1 Coffee plants (*Coffea arabica* L.) growth upon *Funneliformis mosseae* inoculation (MYC) vs the nonmycorrhizal control (NM) under controlled conditions in 'Catimor Amarillo' and 'Typica National' genotypes at the vegetative stage (5 months from inoculation). (a) Graph showing biometric parameters measured across three independent trials (T1, dots; T2, triangles; T3, squares); error plots show the mean value ± the 95% confidence level ($n = 18$). (b, c) Pictures showing the arbuscular mycorrhizal-induced growth effect in 'Typica' (b) and 'Catimor Amarillo' (c) genotypes (Bar, 4 cm). (d) Photosynthetic pigments parameters measured on a fully expanded leaf in Trial 2. Box plots display the median (horizontal line), the quartiles (boxes) and 1.5 interquartile range (whiskers; $n = 6$). Asterisks indicate significant differences among MYC and nonmycorrhizal conditions (Kruskal–Wallis): *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. RFW, root fresh weight; SFW, shoot fresh weight; SL, shoot length.

abundance (a%) close to 100% (Fig. 2a). In line with this, the expression of well-known arbusculated-cell marker genes, that is those involved in lipid (FatM) metabolism (Bravo *et al.*, 2016) and AM-mediated Pi acquisition (PT4; Javot *et al.*, 2007), showed high level of induction upon mycorrhization in both cultivars, indicating a proper arbuscule functioning (Fig. 2b).

According to Shishkoff (1987), coffee roots are characterised by a dimorphic hypodermis which consists of impermeable suberised cells and nonsuberised 'passage cells', which allow the movement of liquids and solutes. In the methyl blue-stained roots of MYC and NM coffee samples, the passage cells appear heavily blue-stained, as in Sharda & Koide (2008), giving the root segments a chessboard appearance (Fig. 2c). Under UV light, transverse sections revealed a strong autofluorescence of hypodermis cell walls (Fig. S3d), as in *Allium porrum* roots (Bonfante-Fasolo & Vian, 1989). The AM fungus produced swollen and lobed hyphopodia on the epidermis (Fig. S3a), but the successful colonisation seemed to be limited to the presence of the passage cells, (Fig. S3b) as in other plants (Liu *et al.*, 2019; Wang *et al.*, 2022). Once it reached cortical cells, the AM fungus produced intracellular coils with limited hyphal branching (Fig. 2d). The fungus moved from one cell to the next, revealing a strong constriction of its hyphal diameter, as in the *Paris-type* colonisation pattern (Fig. S3c).

Transcriptomics offers an overview of coffee responses to the AM fungus

A genome-wide transcriptome analysis was performed on root and leaf tissues of the Typica genotype under the growth conditions described above and 5 months after the fungal inoculation (T1). Typica genotype was chosen since, irrespectively of being more susceptible to AM colonisation, it has a more homogeneous breeding history within the *C. arabica* genetic background, and a high-quality chromosome-level genome sequence is available (Zimin *et al.*, 2018).

The overall gene expression pattern across conditions was investigated by PCA (Fig. 3a). It demonstrated that the principal component 1 (PC1, *x*-axis, 71.93% of explained variance) mainly separates roots from leaves samples, while the second PC (PC2, *y*-axis, 12.25% explained variance) highlighted the effect of mycorrhization. The separation between MYC and NM samples was more evident in the root, where inter-sample variations were lower, while transcriptome pattern between conditions was more similar in leaves, as also shown by distance-based metrics (Fig. S4). The same pattern emerged from differential expression analysis in MYC vs NM samples in both tissues: a large number of transcripts was induced by mycorrhization in roots (4644 isoforms corresponding to 4061 coding gene IDs) while only 1503 (corresponding to 1431 coding gene IDs) in leaves (Dataset S1). In both contrasts, we found a higher amount of upregulated transcripts (Fig. 3b) and a shared core of 406 AM-responsive DEGs in both tissues (Fig. 3c). The analysis was experimentally validated on a different set of samples (Trial 2) by real-time PCR showing good differential expression correlation (Fig. S5).

Functional enrichment analyses were performed using Gene Ontology (GO) and KEGG databases on all the differentially expressed genes. Results showed that 69 and 39 GO categories and 25 and 14 KEGGs categories were enriched in root and leaf, respectively (Dataset S2). The *z*-score values (ratio between up- and

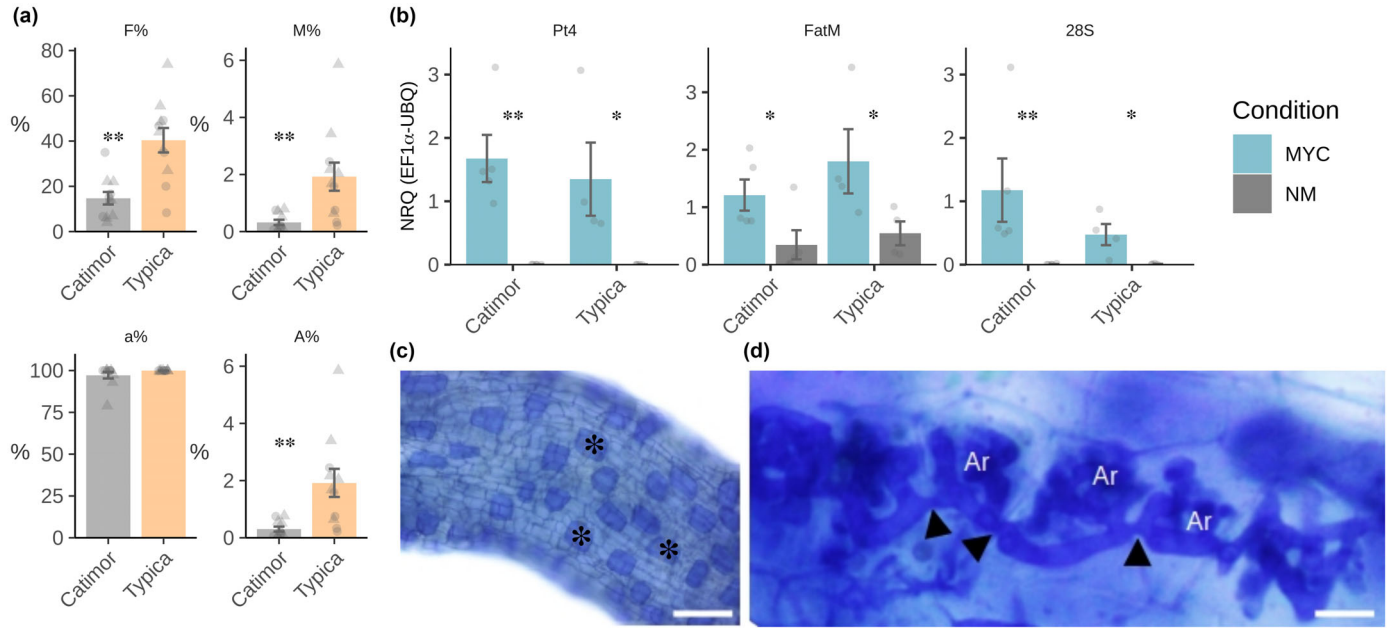

Fig. 2 Arbuscular mycorrhizal colonisation phenotype and functioning of 'Catimor Amarillo' and 'Typica National' *Coffea arabica* L. genotypes inoculated with *Funneliformis mosseae* after 5 months from the inoculation. (a) Graphs show colonisation rate parameters obtained according to the method of Trouvelot *et al*. (1986), measured across three independent trials (T1, dots; T2, triangles; T3, squares); bar plots show the mean value ± SE ($n = 11$); frequency of mycorrhization (F%), intensity of mycorrhization (M%), arbuscules frequency in mycorrhized roots (a%) and frequency of arbuscules in the whole root apparatus (A%) are reported. (b) RT-qPCR gene expression analyses of Pt4 and FatM AM-marker genes. The expression of a *F. mosseae* rRNA marker (28S) was tested to check absence of fungal contamination in nonmycorrhizal (NM) plants (gene details and primer in Supporting Information Table S2); bar plots show the mean value ± SE ($n = 5$), dots represents single replicate value. Asterisks indicate significant differences among mycorrhizal (MYC) and NM conditions (Kruskal–Wallis test): *, $P < 0.05$; **, $P < 0.01$. (c, d) Coffee roots stained with methyl blue and observed under a light microscope. (c) Dimorphic hypodermis of coffee roots composed by impermeable suberised cells and nonsuberised 'passage cells' (asterisks). (d) In colonised root segments, the arbuscules (Ar) are developed in the cortical cells following a 'Paris-type' phenotype: intracellular coils with limited hyphal branching. Arrowheads indicate hyphal constriction points (diameter reduction) between cortical cells. Bars: (c) 100 μm; (d) 20 μm.

downregulated genes in each category) were mostly above zero, indicating a predominance of upregulated DEGs in both plant organs. Among the most-enriched categories in roots (Figs 3d, S6), several functional groups related to primary metabolism, such as membrane processes, transcription machinery (ribosomal proteins), nutrient transport and binding, lipid synthesis, amino acid and glutathione metabolism, and carbon-related pathways, were present. Similarly, primary metabolism was modulated considerably in leaves (Figs 3e, S7): enriched functions included membrane-, carbon- and nutrient-related categories. Sugar synthesis as well as photosynthesis-related pathways were the most relevant categories. The major GO categories were represented by structural components of ribosomes and by translation with 58 and 55 genes, respectively, mostly upregulated. These categories likely reflect the deep cellular reorganisation that root cells undertake at the moment of the fungal entry mirroring what has previously been illustrated by using cell biology approaches (Genre *et al*., 2008; Bonfante, 2018), but also the requirement of a relevant ribosome synthesis (Domingo *et al*., 2023). Finally, the symbiosis's notable impact on secondary metabolism in both roots and leaves was mostly related to stilbenoid and flavonoid pathways.

We predicted orthogroups and ortholog genes in *C. arabica*, *C. eugenioides* and *C. canephora* and four other AM species including *Medicago truncatula* for which the symbiotic genes toolkit is well characterised (Bravo *et al*., 2016; Pecrix *et al*., 2018; Tables S3, S4). The analysis revealed that the majority of AM-specific genes (SymRK, CCaMK, CYCLOPS, RAD1 and STR1-2) were found to be present across all of the coffee species analysed, with some of them exhibiting differential expression in MYC vs NM *C. arabica* roots (Fig. 3f; Tables S4, S5). RAM1, for which no annotated orthologs were found in *C. arabica* genome, represents a relevant exception (Fig. 3f). However, we found a highly similar not-annotated sequence in an unplaced genomic scaffold (Table S6).

In order to validate these results, we next integrated transcriptomics with ion quantification, metabolite identification and quantification, and physiological data. Notably, all these datasets revealed a consistently divergent pattern in MYC and NM plants with a stronger impact in roots than shoots (Fig. 4). In the following section, we discuss the pathways that better support the strong growth effect observed, that is nutrient transport, photosynthetic activity and carbon metabolism, as well as the biosynthesis of secondary metabolites. Other pathways, such as lignin metabolism, development and secondary growth, plant immunity and priming, are commented in Notes S1 and shown in Figs S8–S10.

Multi-omics approaches reveal substantial activation in nutrients acquisition upon mycorrhization

Among the most root-enriched GO terms, a number of membrane-related categories emerged (Figs S6, S7). These

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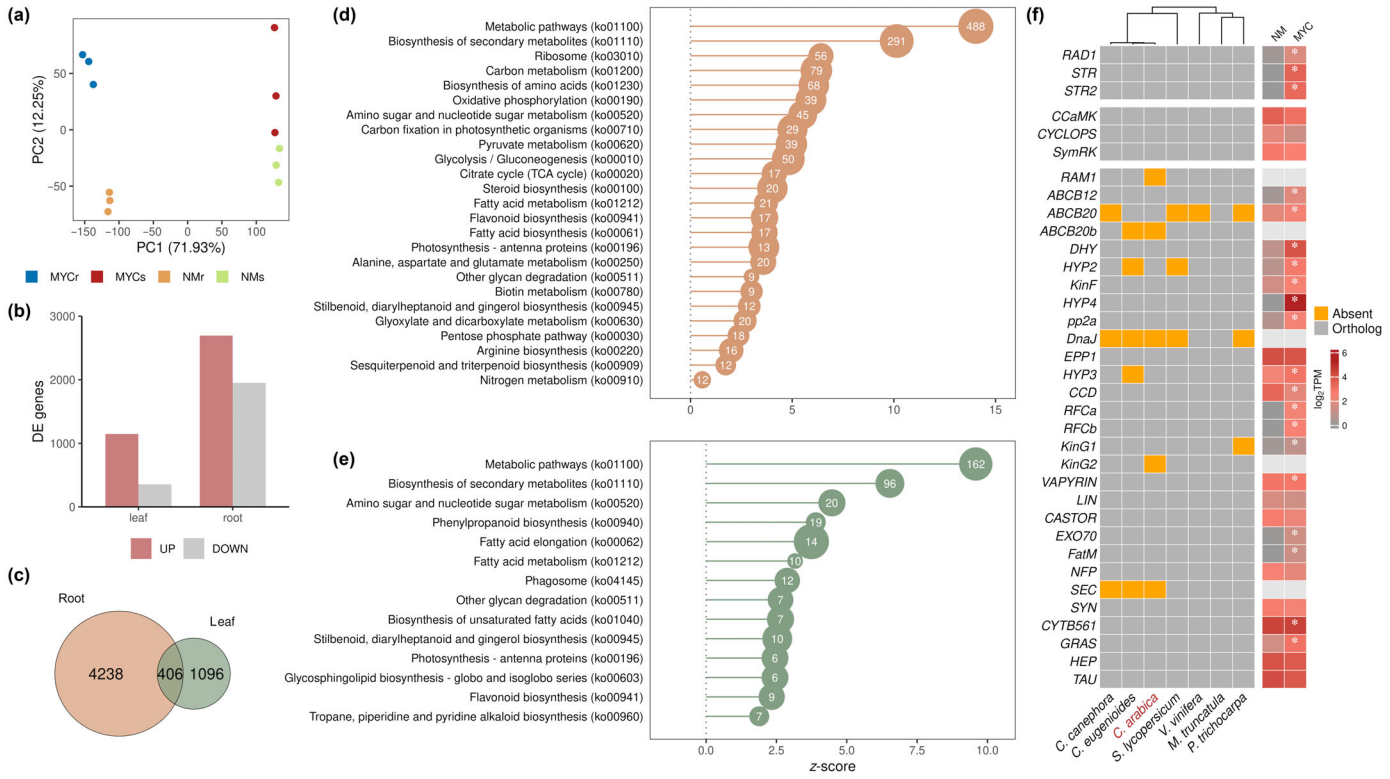

Fig. 3 Genome-wide transcriptome analysis (RNA-Seq) of mycorrhizal (MYC) and nonmycorrhizal (NM) *Coffa arabica* L. plants ('Typica National' genotype) in root (r) and leaf (s) organs. (a) Principal components analysis (PCA) of the transcriptome across conditions and organs. (b) Bar plot showing the number of differentially expressed genes (DEGs) in MYC vs NM contrast in both organs according to up- or downregulation. (c) Venn diagram showing the overlap of DEGs (MYC vs NM) in root and leaf organs. (d, e) KEGG pathways enriched ($P < 0.05$) in root (d) and leaf (e) organs (MYC vs NM); lollipop plots show the amount of DEGs in each category (number in the bubble); the dot radius is $-\log_{10}$-proportional to the enrichment test FDR, while the *z*-score value indicates the dominance of up- (if above 0), or downregulated genes (if below 0) for each category. The top 25 most-enriched categories are shown (all entries for the leaf dataset). (f) Presence of arbuscular mycorrhizal (AM) symbiosis-conserved orthologs (Bravo *et al*., 2016) in *Coffea* sp. and other four AM-competent plant model species. The represented cladogram was inferred on all the predicted orthogroups using Species Tree inference from All Genes (STAG) and shows the theoretical plant phylogeny. The heatmap on the right shows the log-normalised expression level (TPM) of AM-conserved genes in *C. arabica* roots under MYC and NM conditions. Values for each gene represent the mean value of *C. arabica* transcripts which showed orthology to *Medicago truncatula* sequences; asterisks indicate DEGs in the MYC vs NM contrast in *C. arabica*.

categories encompass hundreds of genes upregulated in MYC vs NM, many of which are orthologs of well-known AM-responsive genes including nutrient transporters (ion/cation, amino acid/peptide and sugar transporters) and aquaporins. To support this evidence, we measured major inorganic cation and anion levels of MYC and NM 'Typica' leaf tissue, given that this is the plant organ to which mineral nutrients are eventually translocated, stored, or mainly metabolised. Following this analysis, a PCA ordination revealed divergent anion and cation profiles in MYC and NM plants (Fig. 4a,b). While cation distribution is illustrated in Notes S1, here, the pattern of the major anions is reported integrating all the omics analyses.

Phosphorus Under MYC conditions, we found the upregulation of two homeologs (LOC113709040 and LOC113710656) showing orthology to the mycorrhiza-inducible PT4/PT11 phosphate transporter, which is well-characterised in many plant models (Javot *et al*., 2007; Yang *et al*., 2012). These two transcripts showed high similarity at transcript/protein level (98.55% and 97.73% full-length sequence identity, respectively) and were both upregulated (Table S7). Intriguingly, they are located on two different chromosomes, 9c and 9e, which derive from the two *C. arabica* parental species (*C. canephora* and *C. eugenioides*, respectively) involved in the natural allopolyploidisation event (Scalabrin *et al.*, 2020). Due to their high sequence identity, we were able to validate their cumulative expression by qRT-PCR confirming an almost exclusive expression in MYC roots in both genotypes (Fig. 2b). However, significant mycorrhiza-induced increase in the Pi content of roots and leaves was not observed (Figs 4g, S11). Due to the high rate of primary metabolism in both roots and leaves of MYC plants, we postulate that Pi is immediately used in metabolic pathways.

Sulphur Ion analysis clearly highlighted a significantly higher sulphate anion (SO_4^{2-}) level in MYC plants ($P < 0.05$), which led to a low S-index value suggesting a better S nutritional status compared with NM plants (Fig. 4c). S-index ($([Cl^-] + [NO_3^-] + [PO_4^{3-}]):[SO_4^{2-}]$ ratio) is an indicator of S nutrition according to Etienne *et al.* (2018) and reveals how

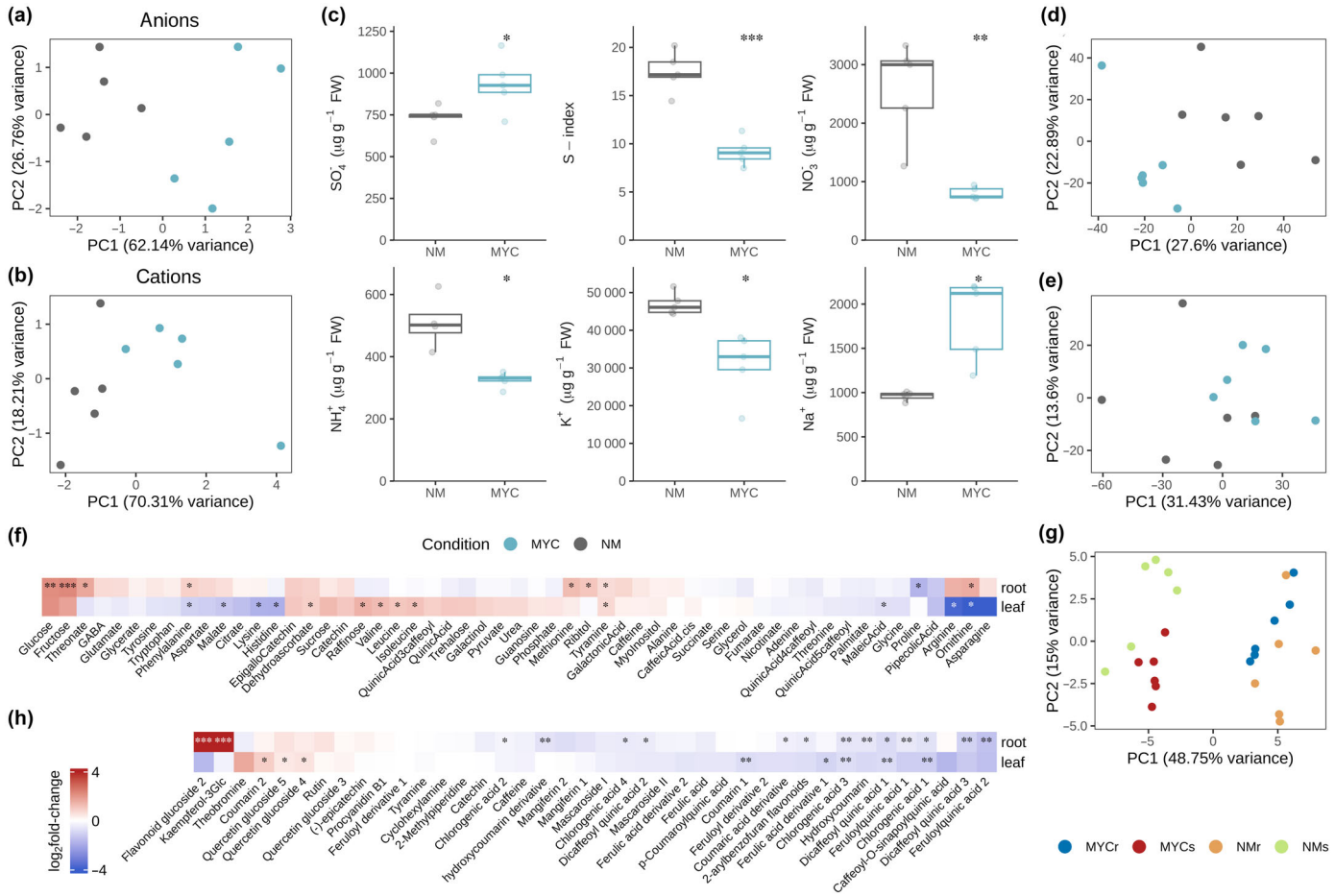

Fig. 4 Ions and metabolome variation in mycorrhizal (MYC) vs nonmycorrhizal (NM) *Coffea arabica* L. plants ('Typica National' genotype) in root (r) and leaf (s) organs. (a, b) Principal components analysis (PCA) of the leaf anions (a) and cations (b) across conditions. (c) leaf ion concentration in MYC and NM samples; box plots display the median (horizontal line), the quartiles (boxes) and 1.5 interquartile range (whiskers; $n = 5$). (d, e) PCA plot of LC–MS dataset in root (d) and leaves (e). (f) Heatmap plot showing $\log_2$fold-change values (MYC vs NM) of metabolites detected in GC–MS analysis. (g) Principal component analysis plot of GC–MS dataset across organs and conditions. In LC–MS dataset, root and leaf data have been normalised separately since they held divergent profiles. (h) Heatmap plot showing $\log_2$fold-change values (MYC vs NM) of metabolites detected in LC–MS analysis. Asterisks indicate significant differences among MYC and NM conditions (Student's *t*-test): *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Sulphur availability affects the inorganic anion balance in plants. These data are in line with the massive upregulation of 11 sulphate transporter genes in MYC roots ('Sulphate transport category', GO:0008272, is also enriched). Among them, we found a putative ortholog (LOC113713553) of the mycorrhiza-induced SULTR1;2 transporter of *Lotus japonicus* (Giovannetti *et al.*, 2014), two isoforms of a low-affinity sulphate transporter (LOC113714900) and a putative ortholog of SULTR2;1 characterised to transfer sulphate into xylem for root-to-shoot transport in Arabidopsis (Maruyama-Nakashita *et al.*, 2015).

Nitrogen A decreased level of both ammonia (NH_4^+) and nitrate (NO_3^-) was observed in MYC plant leaves (Fig. 4c). However, the root transcriptome dataset reported the mycorrhizal-responsiveness of four genes encoding ammonium transporters (all upregulated) and 32 NRT1/PTR family (NPF) proteins. These latter are multisubstrate transporters, including nitrate (Corratgé-Faillie & Lacombe, 2017). Similar to PT4, we found two homeologs of the AM-inducible ammonium transporter 2 (Gutjahr *et al.*, 2009) and two homeologs of the AM-inducible NPF4.5 nitrate transporter (Wang *et al.*, 2020). All these genes were upregulated (Table S7). Due to their very high sequence identity (99.49%), they likely derive from the two *C. arabica* parental species (located on chromosomes 2c and 2e), as discussed for the Pi transporter. Other nitrate transporters showed an opposite trend of expression, being mostly downregulated. The upregulation in MYC roots of glutamine synthase encoding genes (Table S7) supports the hypothesis of an improved nitrate and ammonium assimilation in MYC roots. In leaves of MYC plants, there was additionally a balance between up- and downregulation of NRT1/PTR genes.

Primary and secondary metabolism in MYC vs NM coffee plants

The transcriptome analyses demonstrated that primary metabolism is highly modulated by AM inoculation with changes in KEGG pathways involved in sugar metabolism, amino acids

and fatty acids biosynthesis in root (Fig. 3d). Moreover, KEGG enrichment analysis on DEGs identified biosynthesis of secondary metabolites category (ko01110) as the third and second most represented category in MYC roots and leaves, respectively. We found a strong modulation of several pathways, especially those leading to aromatic compounds and lignin precursors, such as phenylpropanoid (ko00940; Note S1) and flavonoid biosynthesis (ko00941). However, the measurements of total phenols and lignin content (Figs S12, S13) showed no differences between MYC and NM plants, with the exception of 'Catimor' roots where a significant increase in phenolic compounds was observed.

To better understand the transcriptomics and biochemical data, metabolomics was performed by coupling targeted GC–MS and LC–MS analyses (Dataset S3) to broadly cover primary and secondary metabolisms, respectively.

Principal component analysis ordination of the primary metabolome data, targeted to 55 different compounds, was consistent with transcriptomics, with the clearest separation between MYC and NM samples in roots (Fig. 4d,e). Looking at the detected metabolites, different patterns emerged for leaves and roots in response to mycorrhization (Fig. 4f). A significantly higher amount of sugars (glucose and fructose) and other saccharides (ribitol and raffinose), threonate and amino acids (methionine, phenylalanine ornithine and tyramine) was found in MYC roots (Fig. 4g). The profile of primary metabolites induced by AM symbiosis was slightly different in leaves: while valine, leucine and isoleucine amount increased upon mycorrhization, phenylalanine, lysine, histidine, arginine and ornithine decreased (Fig. 4f).

Untargeted metabolomics using LC–MS led to the identification of 40 high-confidence metabolites. Principal component analysis of this dataset (Fig. 4g) detected a stronger impact of the AM symbiosis on the root than on the leaf metabolites.

Since relatively few metabolome data on coffee plants are available, we built a comprehensive secondary metabolite database on this species collecting data from previous work (Martins *et al.*, 2014; Pérez-Míguez *et al.*, 2020; Rocchetti *et al.*, 2020; Grohar *et al.*, 2021; Quintero *et al.*, 2022) to guide the annotation of untargeted analysis. We detected many well-represented compounds including flavanols and flavonols glycosides. In roots, a significantly higher amount of flavonoid- and kaempferol-glycosides emerged (Fig. 4h), while in leaves, only one coumarin and two quercetin glycosides showed higher abundance in MYC plants. This trend towards the production of glycosylated flavonoids well-correlates with the transcriptional activation of the flavonoid biosynthesis pathway (ko00941). By contrast, the LC–MS analysis detected a number of phenolic acids which showed decreased abundance in MYC plants, such as chlorogenic and dicaffeoylquinic acid.

The AM fungus improves coffee photosynthetic efficiency

Both the coffee cultivars revealed greater biomass in their epigeous organs (Fig. 1) opening the question as to whether photosynthesis was the first process to support coffee growth following mycorrhizal colonisation. Transcriptomics showed the enrichment of specific GO/KEGG functional categories in the leaf including photosynthesis light harvesting (GO0009765) and antenna proteins (ko00196), encompassing light-harvesting protein complex of the photosystem II (LHCII), showing all upregulation.

Given that the quantification of photosynthetic pigments and SPAD index revealed increased values in MYC plants (Fig. 1a,d), the functionality of PSII was tested by performing a pulse-amplitude modulation (PAM) fluorimetry analysis. The analysis showed that in both genotypes the F_v/F_m parameter, considered a sensitive indicator of photosynthetic apparatus health (Maxwell & Johnson, 2000; Jägerbrand & Kudo, 2016), was significantly lower in NM plants, while in the MYC ones, it had optimal values (*c.* 0.8, Fig. 5a). MYC plants had also a higher photosystem II (PSII) operating efficiency (Y(II)) and electron transport rate (ETR) than control plants, as well as a lower reduction state of plastoquinone pool ($1\text{-}q_L$; Fig. 5b–d).

All this evidence, combined with the lower concentration of chlorophyll a (Fig. 1d), indicated the loss of reaction centres in NM plants, that is a reduction in the total energy processed through the photochemical pathway. Considering the partitioning of absorbed excitation energy by PSII (Fig. 5e), data showed that the yield of nonphotochemical quenching (YNPQ) was similar under all conditions. However, the Y(NO) parameter, that is the energy passively dissipated in form of heat and fluorescence, was lower for MYC plants. This indicated that NM plants had a greater portion of energy absorbed that was not controlled and dissipated as fluorescence or heat, increasing the possibility of photo-inhibitory damage. Comparing the two cultivars, all these data suggest that differences between MYC and NM were more relevant in Catimor than in Typica cultivar, as also indicated by its enhanced biomass production (Fig. 1a).

Gas exchange measurements highlighted low values of absolute net photosynthetic rate (A) on each condition (see Methods S1). However, MYC plants demonstrated to mitigate the effects of this nutritional limitation on photosynthesis showing higher A than NM plants, even if values were significantly different in the Catimor cultivar only (Fig. 5f). Interestingly, we observed a lower CO_2 concentration of the substomatal chamber (C_i) in MYC plants (Fig. 5i), likely reflecting a higher atmosphere-mesophyll gradient, due to the higher CO_2 assimilation of MYC plants. Transpiration (E) and stomatal conductance (G_s) were unchanged in all treatments and cultivars (Fig. 5g,h) suggesting that stomatal and/or hydraulic regulations were not affected by the symbiotic status (Morte *et al.*, 2000).

Discussion

Since there is a strong interest in traceability and transparency among coffee consumers in line with an increasing attention to more sustainable cultivation approaches, here, we investigated how two *C. arabica* varieties ('Typica National' and 'Catimor Amarillo') respond to the inoculation of an AM fungus under controlled low phosphate conditions. The presence of AM fungi in the roots of coffee plants has already been documented in many areas of cultivation, but the fungal impact on coffee

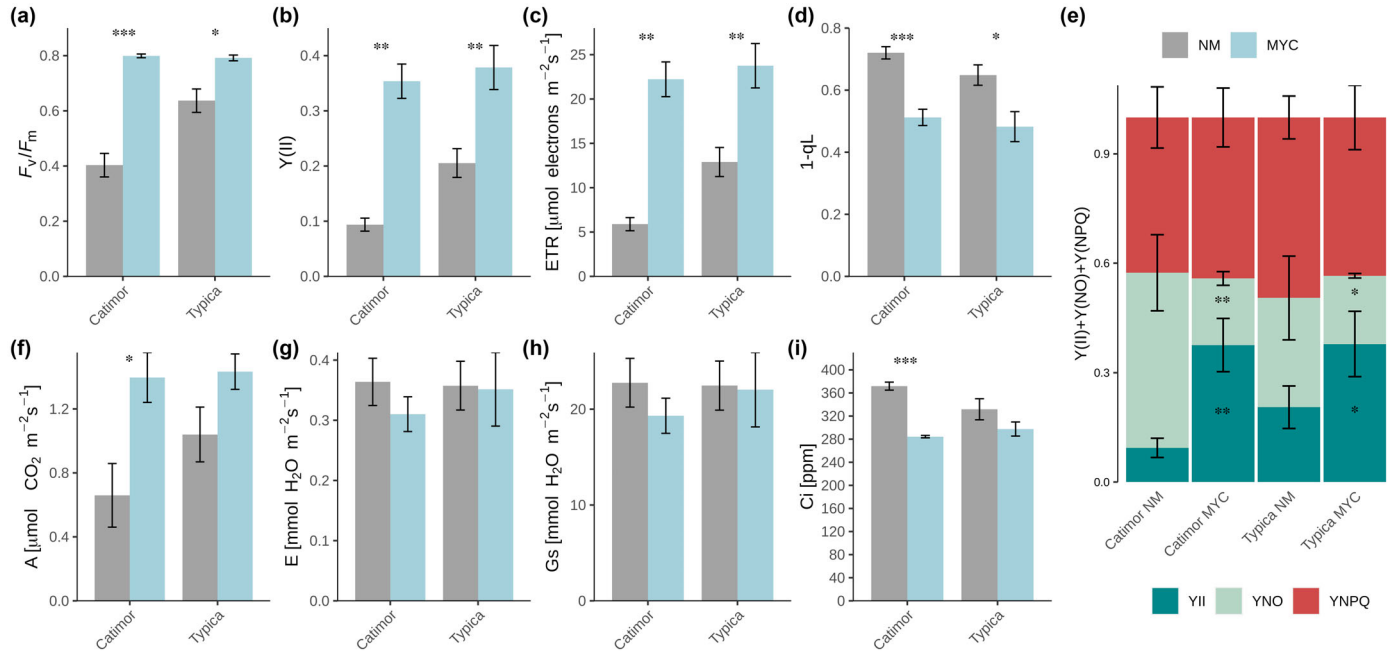

Fig. 5 Photosynthetic and ecophysiological traits of 'Catimor Amarillo' and 'Typica National' *Coffea arabica* L. plants under mycorrhizal (MYC) and nonmycorrhizal (NM) colonisation (5 months from inoculation). (a) Maximum efficiency of photosystem II (PSII) (F_v/F_m), (b) PSII operating efficiency (Y (II)), (c) relative electron transport rate (ETR) and (d) reduction state of plastoquinone pool (1-qL) of leaves were measured by pulse-amplitude modulation (PAM) fluorometry. (e) Partitioning of absorbed excitation energy in PSII. YII, quantum yield of photochemical energy conversion in PSII; YNPQ, quantum yield of regulated nonphotochemical energy loss in PSII; YNO, quantum yield of nonregulated nonphotochemical energy loss in PSII. Values were derived from fluorimetric data. (f) Single leaf net CO_2 assimilation (a, g) transpiration (E), (h) stomatal conductance (G_s) and (i) CO_2 concentration of the sub-stomatal chamber (ci) at and 150 PPFD. Plants were exposed to 150 PPFD actinic light at 23°C and 45% RH. Values are means ± the standard error ($n = 5$ plants). Asterisks indicate statistically supported differences between conditions according to the Student's t-test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

molecular reprogramming has never been experimentally investigated.

In this work, we demonstrate that two coffee cultivars respond to an AM fungus (*F. mosseae*), exhibiting a strong growth effect (Smith & Read, 2008), even if with slight differences between the two genotypes, and that the increased plant biomass is independent of the extent of AM colonisation. In all the three experiments, the intensity of mycorrhization was low (*c.* 20–40%) compared with that detected in other *C. arabica* cultivars (*c.* 40–90%) growing in glasshouse or field conditions (Al-Arequi *et al.*, 2013; Mahdhi *et al.*, 2017; Fonseca *et al.*, 2019). However, these studies also revealed that colonisation intensity and arbuscule number, quantified by different methods, widely vary across genotypes, geography and soil features. In our experiments, the fungus, probably limited by apoplastic barriers present in the dimorphic hypodermis (Shishkoff, 1987), followed a *Paris*-type pattern with mostly intracellular hyphae, as in *Erythronium americanum* (Brundrett & Kendrick, 1990), and carrot (Genre *et al.*, 2008). *F. mosseae*, however, colonised the cortex of a limited fraction of coffee roots. We found that *C. arabica* genome seems to lack an annotated RAM1 gene, which is crucial for the colonisation process (Gobbato *et al.*, 2012). However, some AM-competent species lack RAM1 too, for example *Ginkgo biloba* and *Marchantia paleacea* (Radhakrishnan *et al.*, 2020), suggesting that RAM1 absence is not the first reason for the limited AM colonisation.

Taken in the whole, by using a combination of 'omics', physiological and biochemical approaches, we demonstrate that the patchy fungal presence was sufficient to elicit local and systemic responses.

The AM fungus leads to an improved plant nutrition and photosynthetic efficiency

Arbuscular mycorrhizal symbiosis is known to modulate many metabolic pathways in plant models (Wang *et al.*, 2017), with a stronger impact in roots than in leaves (Fiorilli *et al.*, 2018). Here, transcriptomics revealed that, irrespectively of its shrubby nature, coffee upregulates genes encoding mineral transporters (P_i, NO_3, NH_4, SO_4 and K). We detected that some of these genes were present in two copies (homeologs) in agreement with the *C. arabica* allotetraploid origin. It is known that polyploidy could affect gene expression (Bottani *et al.*, 2018); based on our datasets, we suggest that the two homeologs of AM-inducible phosphate, nitrate and ammonium transporters are both expressed at similar levels.

Transcriptomics, inorganic ion quantification and metabolomics detected a complex scenario in nitrogen metabolism revealing that the MYC coffee plants mainly acquire nitrogen as ammonium and nitrate via a mycorrhizal-dependent pathway (Gutjahr *et al.*, 2009; Wang *et al.*, 2020). Both inorganic N forms can be assimilated to glutamine in roots, explaining their

decreased content in leaves. This process should be particularly important for ammonium, which is considered relatively toxic for plant cells. On the whole, the inorganic–organic N root-to-shoot dynamics lead to a positive N balance in leaves, as revealed by SPAD results and by the increased amino acids content (Fig. 4g). The accumulation of methionine and ornithine mirrored the improved N nutritional status: ornithine, as a nonproteinogenic amino acid and precursor of polyamines, is directly involved in the urea cycle and essential in regulating plant growth, productivity and disease resistance (Majumdar *et al.*, 2013; Hussein *et al.*, 2019). Indeed, a boost in polyamine biosynthesis has been related to increased N and C assimilation by cells (Majumdar *et al.*, 2016).

This improved N nutrition status leads to a general increase in amino acid and protein biosynthesis, which puts its basis on the massive synthesis of ribosomal proteins. A meta-analysis of proteomics datasets on AM plants has indeed demonstrated that the ribosomal translational apparatus has a dominant role in the plant metabolic reprogramming upon AM colonisation (Domingo *et al.*, 2023). The activation of ribosomal machinery seems to be the true core of plant responses, likely supporting the cellular reorganisation and metabolic reprogramming occurring in mycorrhizal plants (Bonfante, 2018).

Taken together, these results reveal that the AM fungal colonisation has a strong positive impact on the mineral nutrition of coffee plants and suggest that it promotes an accelerated uptake, assimilation and circulation of nutrients compared with control plants. The fungus seems to activate mycorrhizal pathways which, following the release of the nutrients by the fungus towards the plant, elicit a range of metabolic routes both at local and systemic levels.

In addition to these responses, coffee plants activate the photosynthetic process by increasing the synthesis and accumulation of photosynthetic pigments, the expression of genes relevant for both light and dark reactions, and the efficiency of some steps, such as a decreased energy loss as heat.

Many studies have demonstrated that mycorrhization has an impact on the photosynthetic process: a meta-analysis (Chandrasekaran *et al.*, 2019), mostly focussed on the increase in photosynthesis in plants under salt stress, correlated the higher photosynthetic activity of AM plants with a higher chlorophyll content and detected many differences in the ecophysiological parameters when they compared mycorrhizal C_3 and C_4 plants. Similarly, in another meta-analysis focussed on the behaviour of photosystem II, Wang *et al.* (2019) detected many differences between dicots and monocots and, interestingly, found that *Funneliformis mosseae* was the most effective AM fungus in enhancing the plant photosynthesis performance. Looking at the underlying molecular mechanisms, many investigations have revealed an upregulation of photosynthetic genes in different crops such as tomato and wheat (Zouari *et al.*, 2014; Fiorilli *et al.*, 2018). However, only Gerlach *et al.* (2015) tried to correlate the photosynthetic behaviour of maize integrating transcriptomic, metabolomics and ecophysiological analysis. In contrast to our findings, they report that many photosynthesis-related genes, as those coding for proteins of the light-harvesting complex or for key enzymes such as ribulose bisphosphate carboxylase/oxygenase, were mostly repressed in mycorrhizal samples (Gerlach *et al.*, 2015).

The photosynthetic process of MYC plants also led to an increased root carbon sink with the accumulation of glucose and fructose. The AM fungus may trigger such sugar mobilisation (Gutjahr *et al.*, 2009), but due to the low fungal colonisation, the sugar excess is probably accumulated as soluble sugars. As expected, our transcriptomics data showed that genes coding for starch synthesis, as starch synthase, were mostly downregulated in roots of MYC plants, confirming the long-dated knowledge that starch is not accumulated in arbusculated cells (Gutjahr *et al.*, 2009).

This set of data offers new ideas to explain the impact of AM fungi on the root–shoot axis which links the two crucial functions of a plant, that is mineral nutrition in the root and sugar production in the leaf. The molecular signal which leads to the photosynthetic activation upon AM colonisation is unknown, but chitin oligomers, well-known bioactive molecules released by the AM fungi and perceived by the plant (Genre *et al.*, 2013; Volpe *et al.*, 2023), have been demonstrated to also elicit photosynthesis in wheat (Li *et al.*, 2020).

AM symbiosis modulates lipid and secondary metabolism in coffee plants

The investigation of other AM-modulated metabolic pathways confirmed previous knowledge on AM plants: the AM fungus, which is lipid auxotrophic, elicits lipid biosynthesis (Luginbuehl *et al.*, 2017; Venice *et al.*, 2021) as well as a general antioxidant status, which in coffee plants is well-supported by the activation of glutathione metabolism and by the enzymes involved in reactive-oxygen species response, as already demonstrated in other plants (Hu *et al.*, 2020; Venice *et al.*, 2021). These data allow us to conclude that perennial and annual plants set up similar responses to AM fungi. Most of our knowledge on AM functionality stems from annual plants with a few exceptions (Balestrini *et al.*, 2017). Notwithstanding the genetic and metabolic differences, related to the secondary growth, AM fungi are exclusively present in roots with a primary body, eliciting a molecular response which largely overlaps with that activated in annual species.

Lastly, coffee is so precious due to the production of a fruit rich in many important secondary metabolites. Transcriptomics identified the category of secondary metabolites as one of the most differentially expressed upon mycorrhization, together with phenylpropanoid biosynthesis. The latter indicates an upregulation of the lignin synthesis which has already been detected in some AM plants (Chialva *et al.*, 2018; Tian *et al.*, 2021; Venice *et al.*, 2021). The metabolome profiling performed in this study has identified a number of flavonols (mostly found in their *O*-glycoside form), which hold well-known antioxidant and neuroprotective properties in human diet (Barreca *et al.*, 2021), to be induced by mycorrhization. Flavonoid metabolism shares many of its early biosynthetic genes with the phenylpropanoid pathway. Transcriptomics, metabolomics and biochemical analyses revealed some discrepancies in the metabolite dynamics

(Notes S1; Figs S12, S13): they can be partly explained by the common origin of flavonoids and lignin synthesis pathways which originate from 4-coumaroyl-CoA and therefore share a number of intermediates. An inverse correlation has been reported for chlorogenic acid and lignin synthesis in Arabidopsis, being both products of the phenylpropanoid pathway (Volpi e Silva *et al.*, 2019). In our datasets, chlorogenic acid, which plays a potential role in stress response, was induced by mycorrhization, while lignin, which has a structural function, showed similar concentrations in MYC and NM samples. Our analysis suggests a comparable potential interdependence between the two coffee metabolic routes, leading to a more consistent presence of flavonoids than lignin precursors in MYC plants.

In the whole, omics highlighted the increased abundance in MYC plants of flavonol glycosides, which are also well known for their antioxidant and protecting properties against UV radiation and osmotic stress (Jańczak-Pieniążek *et al.*, 2021; Singh *et al.*, 2021). The synthesis of secondary metabolites has been traditionally described as one aspect of the multiple plant responses to pathogens (Bennett & Wallsgrove, 1994; Piasecka *et al.*, 2015). Arbuscular mycorrhizal fungi are well known to induce a priming response with the activation of plant genes which are related to pattern-triggered immunity response (Pozo & Azcón-Aguilar, 2007; Chialva *et al.*, 2018; Vannini *et al.*, 2021). Even if such a response is activated in the coffee transcriptome (Fig. S9; Notes S1), we suggest that the improved nutritional status could be one of the first factors to elicit the activation of secondary metabolisms.

In conclusion, we presented a set of congruent transcriptomics, metabolomics and physiological data demonstrating that an AM fungus improves plant biomass in two coffee cultivars, reprogramming many different primary metabolic pathways which offer a new light to the mycorrhizal root–shoot axis. On the one hand, the AM fungus promotes the uptake and circulation of nutrients in the mycorrhizal plants, and on the other hand, it has an impact on the photosynthetic process, since the symbiotic status activates the light-harvesting system making PSII more performing in all its photochemical features. Similarly, mycorrhizal plants activate their secondary metabolism leading to an increased quantity of flavanols. However, the impact on the synthesis of these secondary metabolites is stronger at local level, opening the question whether the symbiosis may also have an impact on their content in seed and fruit.

Irrespective of these crucial questions, which requires more focused studies under different conditions, the clear-cut positive effect of the AM symbiosis on coffee plant performance suggests its relevance to enhance seedling fitness/survival in orchards. Improving coffee plant production in nurseries would dramatically improve future plant productivity (WCR, 2022) in the perspective of a more ecologically sustainable cultivation.

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Competing interests

None declared.

Author contributions

PB, MC and VF designed the research. MC, DP, MN, LPS, SV and M Maghrebi performed the experiments. MC, DP, MN, LPS and M Maghrebi collected data and performed formal data analysis. M Morgante analysed genomes of the parental cultivars. MC, PB, DP, VF and GV interpreted data. MC and MN performed the visualisation. PB and MC wrote the original draft. All the authors contributed to the review and editing of the manuscript. LL, VF, ARF, GV and CL acquired the funding.

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Data availability

The data that support the findings of this study are openly available in the NCBI Sequence Read Archive (SRA) under BioProject accession no PRJNA908529 and in FigShare at doi: 10.6084/m9.figshare.21667106.

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Supporting Information

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

Dataset S1 Differentially expressed genes in root and leaf organs of mycorrhizal vs nonmycorrhizal (NM) *Coffea arabica* cv 'Typica National' plants.

Dataset S2 Gene Ontology and KEGG pathway functional enrichments (FDR < 0.05) of differentially expressed gene lists (mycorrhizal vs nonmycorrhizal) in *Coffea arabica* cv 'Typica National' plants.

Dataset S3 Normalised metabolites data from GC–MS and LC–MS analysis in roots and leaves of mycorrhizal and nonmycorrhizal *Coffea arabica* cv 'Typica National' plants.

Fig. S1 Coffee (*Coffea arabica* L.) plant growth effect upon *Funneliformis mosseae* inoculation vs the nonmycorrhizal control under controlled conditions in 'Catimor Amarillo' and 'Typica National' genotypes at the vegetative stage (5 months from inoculation).

Fig. S2 Increase in root and shoot dry weights and internodes and leaves number in *Coffea arabica* plants ('Typica National' and 'Catimor Amarillo') inoculated with *Funneliformis mosseae* (MYC), compared with the nonmycorrhizal controls (NM).

Fig. S3 Details of the arbuscular mycorrhizal fungal colonisation in *Coffea arbica* roots.

Fig. S4 Sample-to-sample distance heatmap of RNA-Seq libraries.

Fig. S5 Linear correlation between differential expression values (log_2fold-changes) of mycorrhizal vs nonmycorrhizal *Coffea arabica* cv 'Typica' roots from RNA-Seq experiment (DEGs) and RT-qPCR data obtained on a different experimental sample set on both 'Typica' and 'Catimor' cultivars.

Fig. S6 Top 25 most-enriched ($P < 0.05$) Gene Ontology terms among mycorrhizal vs nonmycorrhizal differentially expressed genes in *Coffea arabica* roots.

Fig. S7 Top 25 most-enriched ($P < 0.05$) Gene Ontology terms among mycorrhizal vs nonmycorrhizal differentially expressed genes in *Coffea arabica* leaf.

Fig. S8 Transcriptome modulation of the phenylpropanoid biosynthesis KEGG pathway (ko00940) in mycorrhizal vs nonmycorrhizal *Coffea arabica* cv 'Typica National' plants.

Fig. S9 Transcriptome modulation of the plant–pathogen interaction KEGG pathway (ko04626) in mycorrhizal vs nonmycorrhizal *Coffea arabica* cv 'Typica National' plants.

Fig. S10 Transcriptome modulation of the glutathione metabolism KEGG pathway (ko00480) in mycorrhizal vs nonmycorrhizal *Coffea arabica* cv 'Typica National' plants.

Fig. S11 Concentration of other leaf ions measured in *Coffea arabica* cv 'Typica National' leaves in mycorrhizal and nonmycorrhizal samples.

Fig. S12 Total phenols concentrations in *Coffea arabica* cv 'Catimor' and 'Typica National' roots and leaves in mycorrhizal and nonmycorrhizal samples.

Fig. S13 Lignin concentrations in *Coffea arabica* cv 'Catimor' and 'Typica National' roots, leaves and stem in mycorrhizal and nonmycorrhizal samples.

Methods S1 Detailed experimental procedures used to generate molecular, biochemical and physiological data.

Notes S1 Additional information on metabolisms modulated upon mycorrhizal inoculation in the root and leaf of *Coffea arabica* plants.

Table S1 RNA-Seq libraries filtering and mapping statistics.

Table S2 Oligonucleotides sequences used in RT-qPCR experiments.

Table S3 Proteome/Genome versions used for orthogroups inference.

Table S4 Details of arbuscular mycorrhizal symbiosis-conserved gene identified by phylogenomics in Bravo *et al.* (2016) and Radhakrishnan *et al.* (2020) and their orthologies in *Coffea* sp. and other selected models as used in Fig. 3(f).

Table S5 Arbuscular mycorrhizal symbiosis-conserved genes in *Coffea arabica* and their expression level in mycorrhizal and nonmycorrhizal root samples.

Table S6 Sequences collected by BLASTP and TBLASTN against *Coffea arabica* proteome, transcriptome and genome assembly using as a query orthologs of arbuscular mycorrhizal-conserved genes in *Medicago truncatula* and other coffee species, which were not identified in the *Coffea arabica* proteome.

Table S7 Differential gene expression of selected transcripts involved in plant nutrient transport and metabolism in mycorrhizal vs nonmycorrhizal roots of *Coffea arabica* cv 'Typica'.

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