Received: 14 February 2024 | Revised: 29 May 2024 | Accepted: 6 June 2024

DOI: 10.1111/pce.15007

ORIGINAL ARTICLE

Plant, Cell & Environment | WILEY

Genetic and functional traits limit the success of colonisation by arbuscular mycorrhizal fungi in a tomato wild relative

Matteo Chialva | Stefania Stelluti | Mara Novero | Simon Masson | Paola Bonfante | Luisa Lanfranco

Department of Life Sciences and Systems Biology, University of Torino, Torino, Italy

Correspondence
Matteo Chialva and Luisa Lanfranco, Department of Life Sciences and Systems Biology, University of Torino, Viale Mattioli 25, 10125, Torino, Italy.
Email: matteo.chialva@unito.it and luisa.lanfranco@unito.it

Present address
Stefania Stelluti, Department of Agricultural, Forest and Food Sciences, University of Torino, Largo P. Braccini 2, Grugliasco, 10095, Italy.

Funding information
European Commission, Grant/Award Number: 727929; Italian Ministry of University and Research, Grant/Award Number: CN_00000033

Abstract

To understand whether domestication had an impact on susceptibility and responsiveness to arbuscular mycorrhizal fungi (AMF) in tomato (*Solanum lycopersicum*), we investigated two tomato cultivars ("M82" and "Moneymaker") and a panel of wild relatives including *S. neorickii*, *S. habrochaites* and *S. pennellii* encompassing the whole *Lycopersicon* clade. Most genotypes revealed good AM colonisation levels when inoculated with the AMF *Funneliformis mosseae*. By contrast, both *S. pennellii* accessions analysed showed a very low colonisation, but with normal arbuscule morphology, and a negative response in terms of root and shoot biomass. This behaviour was independent of fungal identity and environmental conditions. Genomic and transcriptomic analyses revealed in *S. pennellii* the lack of genes identified within QTLs for AM colonisation, a limited transcriptional reprogramming upon mycorrhization and a differential regulation of strigolactones and AM-related genes compared to tomato. Donor plants experiments indicated that the AMF could represent a cost for *S. pennellii*: *F. mosseae* could extensively colonise the root only when it was part of a mycorrhizal network, but a higher mycorrhization led to a higher inhibition of plant growth. These results suggest that genetics and functional traits of *S. pennellii* are responsible for the limited extent of AMF colonisation.

KEYWORDS
arbuscular mycorrhizal symbiosis, domestication, *Funneliformis mosseae*, mycorrhizal growth response, *Phelipanche*, *Solanum lycopersicum*, *Solanum pennellii*, strigolactones, transcriptomics

1 | INTRODUCTION

Among the crops which feed humans, tomato (*Solanum lycopersicum* L.) is one of the most relevant since its fruit is a source of health-promoting molecules including vitamins, carotenoids, and phenolic compounds (Li et al., 2022). According to FAO, the total world tomato production revealed a weak positive trend in 2022 (189.1 million metric tonnes) when compared to previous years (up 2% when compared to 2020). Tomato cultivation seems in fact to be impacted by the current rising temperatures coupled with water stress. Severe effects on tomato growth and productivity have been reported in Florida which ranks first among US states in fresh-market tomato production (Ayankojo & Morgan, 2020). In Mediterranean areas, where tomato is one of the most extensively cultivated crops, a drastic reduction of rainfall and increased temperatures have already been observed: a warming which is 20% faster than the global average may lead to a predicted loss of 30% yield for mediterranean crops (Saadi et al., 2015). In addition, growth of tomato plants under

elevated carbon dioxide conditions revealed changes in the fruit nutritional profiles (Boufeldja et al., 2022).

Many research have revealed how tomato has lost its genetic diversity during domestication, which led to a genetic bottleneck (Sato et al., 2012), even if the modern artificial selection allowed the development of bigger fruits. By contrast, tomato wild relatives (*Solanum* sect. *Lycopersicon*) still maintain high genetic diversity and have been used as a valuable genetic reservoir for cultivated tomato (Bolger et al., 2014; Castañeda-Álvarez et al., 2016). A recent pangenomic analysis highlights such a potentiality of tomato wild relatives: Li et al. (2023) performed a genome assembly of nine wild species and two domesticated accessions of tomato generating a super-pangenome of the entire tomato clade. Comparative analyses revealed deep genetic differences and led to the discovery of a wild tomato gene, encoding for a cytochrome P450, that has the potential for yield increase in modern breeding (Li et al., 2023). Some of these wild relatives, such as *S. pimpinellifolium*, *S. pennellii*, *S. habrochaites* and *S. chmielewskii*, have also been identified as tolerant to high temperature and drought stress, mostly during seed germination and seedling growth. In a genetic analysis, Pessoa et al. (2023) identified some candidate genes as associated with drought tolerance in *S. pennellii* suggesting that they can be transferred to elite tomato cultivars through marker-assisted breeding technologies.

While these genetic resources have unravelled many aspects of tomato biology, from fruit shape and ripening to resistance to biotic/abiotic stresses, they have been rarely used to investigate tomato responsiveness to Arbuscular Mycorrhizal Fungi (AMF) (Martín-Robles et al., 2018; Plouznikoff et al., 2019). As most land plants living in natural and agricultural environments, tomato hosts AMF in their roots. Thanks to this intimate mutualistic association, AMF develop extraradical hyphae which efficiently harvest mineral nutrients from the soil and deliver them to the host during the intraradical phase. On the other hand, the plant provides the fungus with reduced carbon in the form of sugars and lipids (Genre et al., 2020). Due to the establishment of a root-shoot axis (Chialva et al., 2023), AM symbiosis leads to a systemic effect characterised by an increased shoot biomass (the so-called "growth effect"; Hoeksema et al., 2010) and often by higher resilience to biotic and abiotic stresses (Diagne et al., 2020). However, these effects do not occur in all tomato cultivars (Smith et al., 2003). In addition to the impact of phosphate fertilisers which hamper mycorrhization success, Martín-Robles et al. (2018) suggested that the ancient domestication process and the more recent breeding programs have not considered the beneficial impacts of AMF. They found that the effect of domestication on AM responsiveness across 27 crop species and their wild progenitors varied depending on phosphorus (P) availability: wild relatives benefited from the symbiosis irrespective of P availability, while domesticated crops only profited under P-limited conditions. Domestication might have therefore altered the regulation of resource trafficking between AMF and their plant hosts (Martín-Robles et al., 2018).

When considering the Mycorrhizal Growth Responses (MGR), a meta analysis (Chandrasekaran et al., 2019) across different plant families revealed that plant growth parameters were generally positively affected by the presence of AMF. *Funneliformis mosseae* and *Rhizophagus intraradices* resulted to be efficient symbionts in tomato: they stimulate yields in the Rio Grande and Zelig cultivars, respectively (Fracasso et al., 2020; Leventis et al., 2021; Ullah et al., 2023). However, inoculation with *Gigaspora margarita* led to a limited colonisation and a negative MGR of tomato 'M82' (Chialva et al., 2020). Therefore, domesticated tomato respond differently depending on plant and AMF genotypes. However, AMF responsiveness in tomato wild relatives has never been comprehensively assessed neither at root colonisation level nor at plant systemic responses, with the exception of *S. pimpinellifolium* (Martín-Robles et al., 2018; Plouznikoff et al., 2019).

To fill this gap of knowledge, following the hypothesis that wild relative species were more responsive to AM colonisation, we investigated AMF responsiveness in *S. lycopersicum* and its wild relatives. A panel of wild relatives including *S. neorickii*, *S. habrochaites* and *S. pennellii* was selected and their susceptibility and responsiveness after inoculation with *Funeliformis mosseae* was compared with two *S. lycopersicum* cultivars ('M82' and 'Moneymaker'). These species are well-acknowledged sources of genetic diversity for cultivated tomato and represent different genetic groups in the phylogeny of the clade. Since *S. pennellii* resulted the least responsive genotype to AM colonisation, we focused our investigation on the comparison between *S. pennellii* and *S. lycopersicum* by considering both optimal (well-watered) growth conditions and combined moderate drought stress/nutrient starvation to decipher the molecular basis of wild relatives versus domesticated tomato responses when interacting with AMF.

2 | MATERIALS AND METHODS

2.1 | Plant material growth and sampling

An initial screening was conducted on six cultivar/ecotypes encompassing three tomato wild relative species within the *Solanum* sect. *Lycopersicon* clade, namely *S. habrochaites* (LA1777), *S. neorickii* (LA2133), *S. pennellii* (LA5240, CC2476), and the two tomato (*S. lycopersicum*) cultivars 'M82' and 'Moneymaker'. In the following experiments *S. lycopersicum* cv. 'M82' was selected as a reference tomato genotype and compared with *Solanum pennellii* (LA5240) under arbuscular mycorrhizal (AM) symbiosis. In addition the *S. lycopersicum* x *S. pennellii* F1 hybrid (Torgeman & Zamir, 2023) was also tested.

In all experiments, seeds were surface sterilised in 2.7% bleach and then rinsed in water according to TGRC (Tomato Genetic Resource Center) guidelines and directly sowed in a miniaturised growing system consisting in small alveolar trays with 60 wells (5 x 5 x 14 cm and 100 mL vol) each. Mycorrhizal condition was obtained inoculating a monospecific *Funneliformis mosseae* inoculum purchased from MycAgro Lab (Dijon). Control plants were grown on a similar substrate without AMF spores ("blank inoculum"). Both substrates were 25% diluted in oven-sterilised (3 h at 180°C) quartz sand which allows to finely modulate nutrients introduced into the

system. For each experimental condition 6–12 plants were prepared and grown under controlled conditions (14 h light at 24°C and 10 h dark at 20°C). Mycorrhizal colonisation was also tested with *Rhizophagus irregularis* (DAOM-197198) as symbiont by inoculating 4000 spores per pot using a liquid inoculum (Agronutrition) under the same conditions previously described. In all these experiments plants were grown at low P level (50 µM Na_2HPO_4) using a modified Long-Ashton solution (N, 1 mM and K, 1 mM) to ensure good levels of root colonisation. Combined nutrients and moderate water stress was applied to tomato 'M82' and *S. pennellii* (LA5240) as described in Chialva et al. (2020). Briefly, after the first month in which AM symbiosis could establish without interferences, moderate stress was induced gradually lowering the soil water capacity (SWC) as described in Lehnert et al. (2017). The required moderate water stress conditions were achieved at a stem water potential (WP) between −0.6 and −0.8 MPa, which corresponded to a SWC of 35% according to a previous trial (Chialva et al., 2020) in which different water regimes were applied and stem WP (Ψ_w) measured. Stem WP was indirectly measured from leaves as described in de Freitas et al. (2011). Briefly, leaves were enclosed in plastic bags containing a piece of wet filter paper and a reflective envelope to suppress transpiration allowing the leaf WP to equilibrate with the stem for at least 20 min. Leaves were then excised from the stem and immediately measured using a Schölander pressure chamber. The weight of the system was thus monitored every 2 days to maintain 35% and > 90% of the SWC in combined stress (CS) and well-watered (WW) conditions, respectively. To maintain the SWC at the given levels, plants were only watered with the nutrient solution. The lowering of water provision under CS conditions also reduced the total amount of nutrients provided to the plants (−65%) compared with WW conditions resulting in moderate nutrient stress. At the end of the experiment stem WP was monitored on three randomly-selected individuals for each treatment to confirm water stress regime application.

After 1 month of continuous stress, plants were sampled measuring root fresh/dry weight (RFW and RDW), shoot fresh/dry weight (SFW, SDW) and shoot length (SL). The SPAD index was measured using a SPAD-502 Chlorophyll Meter (Konica-Minolta Inc.) averaging the value of three different leaflets of the first fully expanded leaf from apical meristem for each plant. Mycorrhizal growth response (MGR) was calculated according to Johnson et al. (2015). Total phosphorus content was extracted from oven-dried shoot material (48 h at 40°C) and spectrophotometrically quantified as described in Celi et al. (2013).

2.2 | Quantification and imaging of mycorrhizal colonisation

Mycorrhizal colonisation was confirmed on five plants for each treatment (including non-mycorrhizal controls) staining roots with 0.1% cotton blue (Sigma-Aldrich) and observing at light microscope at least 1 m of roots for each plant according to Trouvelot et al. (1986).

Intraradical fungal structures were stained using Wheat Germ Agglutinin-FITC conjugate (Sigma-Aldrich). Briefly, 5 mm-long root segments surrounded by extraradical mycelium were collected, washed under tap water, fixed overnight at 4°C in 4% para-formaldehyde in PBS buffer (0.05 M, pH 7.2). Roots were washed 3 times in PBS (10 min. each) and included in 8% Agarose Type II-A (Sigma-Aldrich). 100 µm-thick sections were obtained using a vibratome, washed for 5 min in commercial bleach diluted 1:30 in PBS, washed 3 times in PBS (10 min. each) and finally incubated for 2 h in the dark with WGA-FITC conjugate (1 mg/mL). Sections were then rinsed in PBS (3 washes, 10 min. each), mounted on glass slides with the anti-fading buffer and then observed under confocal microscope (Leica TCS-SP2 confocal microscope, Leica Microsystems GmbH). The 543 nm Ar laser band was used to excite the FITC and the fluorescent signal was collected with an emission window at 580–650 nm.

2.3 | RNA-seq data analysis

S. pennellii (accession LA5240) and *S. lycopersicum* 'M82' root tissues obtained under mycorrhizal and non-mycorrhizal inoculation, and under moderate nutrients-water stress as described above were sampled for genome-wide transcriptome analysis. RNA was isolated from freeze-dried roots using NucleoSpin® RNA Plant and Fungi kit (Macherey-Nagel) and spectrophotometrically quantified (Nanodrop Technologies Inc.) checking the A260/280 nm and A260/230 nm ratios. RNA integrity was then checked by capillary electrophoresis using an Agilent 2100 Bioanalyzer instrument with the Agilent RNA 6000 Nano Kit following manufacturer's instructions. RNA materials with a RIN value > 7 were sent to Macrogen for sequencing using the TruSeq RNA Sample Prep Kit v2 and Illumina NovaSeq6000 paired-end sequencing (2 × 100 bp, 30 M reads per sample) following ENCODE standards (https://www.encodeproject.org). Three biological replicates per condition were sequenced, each consisting in a pool of roots of three different plants, resulting in a total of 24 samples.

Libraries were quality-checked using FastQC v0.11.7 software and adapters trimming and quality filtering of reads were performed using BBDuk (https://jgi.doe.gov/data-and-tools/bbtools/) v38.29 using a k-mer size of 23, the built-in Illumina adapters reference database and filtering for read-length > 76 and quality > 15. Filtered reads were then pseudo-mapped on the reference transcriptomes for each species and counted using 'salmon' v0.11.3 (Patro et al., 2017). The SL3.0, ITAG3.2 transcriptome version for *S. lycopersicum* (https://solgenomics.net/) and the cDNAs from the v2.0 genome for *S. pennellii* were used (Bolger et al., 2014).

Downstream analyses were performed in R programming environment v4.3.1 (R Core Team, 2023) on raw counts obtained from salmon software using the 'tximport' R library (Soneson et al., 2015) and collapsing counts from isoforms to genes level. Low-count genes were first filtered using HTSfilter pipeline v1.24.0 (Rau et al., 2013) and unwanted variation (library preparation artifacts, stochastic effects) screened using surrogate variable

analysis (SVA) performed with function 'svaseq' in R package 'sva' v3.32.1 (Leek et al., 2023). Since in both *S. lycopersicum* and *S. pennellii* libraries two surrogate variables were found, unwanted variation was removed from count tables using RUVg normalization method ($k = 2$) in the R library 'RUVSeq' v1.34.0 (Risso et al., 2014) which implements a factor analysis on a pre-defined sets of empirical control genes which are not modulated among experimental conditions (false-discovery rate (FDR) > 0.8 and $\log_2$fold-change $-0.1 < x < 0.1$). Afterwards, differential expression analysis was performed inferring a negative binomial generalized linear model (GLM) as implemented in 'DESeq. 2' v1.24.0 (Love et al., 2014) setting betaprior = T, independentFiltering=F and a FDR threshold of 0.05 including also the 2 surrogate variables detected in the design formula. After DESeq2, 'vst' (variance stabilizing transformation) normalization, sample to sample distances and PCA plot were generated and outliers (2 libraries out of 12 for both species) were removed keeping at least two samples for each treatment according to ENCODE standards. Differentially expression analyses were then performed considering and FDR threshold of 0.05. Variance partitioning analysis (VPA) was performed on the vst-transformed counts table using 'varpart' function in R package 'vegan' v2.6-4 (Oksanen et al., 2022), testing partitions for significance using permutational ANOVA (999 permutations, $p < 0.05$) on the RDA model. Gene-Ontology functional categories were enriched among DEGs using the 'goseq' v1.52.0 package (Young et al., 2010) implementing the built-in transcript length-bias correction at a FDR < 0.1. For each enriched category a z-score indicating the ratio between up- and downregulated genes was calculated using 'GOplot' v1.0.2 package (Walter et al., 2015).

2.4 | Identification of orthologous genes, motif-discovery and protein structure analysis

Orthologous genes between *S. lycopersicum* and *S. pennellii* were identified on full proteomes sequence using OrthoFinder v2.2.7 (Emms & Kelly, 2019) setting 'blast' as a sequence search program, and 'msa' combined with 'fasttree' as gene tree inference methods. The analysis, which accounted for gene duplications across species, identified a total of 24,104 and 29,512 orthologous genes for *S. lycopersicum* and *S. pennellii* respectively, encompassing 20,162 high-confidence one-to-one orthologous pairs.

Cis-regulatory elements in genes promoters (5 kb upstream of the ATG) were searched using the 'dna-pattern' tool in RSAT Plants (available at https://rsat.eead.csic.es/plants/) with default parameters. Visualisation of the motifs pattern in the promoters was performed using the Gene Structure Display Server (GSDS) v2 (Hu et al., 2015).

The comparative analysis of the LYK10 protein structure in *S. pennellii* and tomato was performed using ragp v0.3.5.9000 (Dragićević et al., 2020) R package by searching the protein sequence on Pfam-A database using HMMER, predicting signal peptides with Signalp 5.0 and transmembrane domain with Phobius.

2.5 | RT-qPCR analysis

For real-time PCR experiments, RNA was diluted to 200 ng/µl and subjected to a DNAse treatment using the Turbo DNA-free™ kit (Ambion) according to the manufacturer's instructions. The absence of DNA contamination was assessed in PCR assays using primers for the tomato ubiquitin gene (Supporting Information S1: Table S1). cDNA was synthesised from 250 to 750 ng of total RNA using the Superscript™ II Reverse Transcriptase Kit (Invitrogen) according to the manufacturer's instructions. cDNA was diluted 1:2 with nuclease-free water for quantitative relative expression analysis (RT-qPCR). Reactions were carried in two technical replicates using a Rotor-Gene Q instrument (QIAGEN) in a final volume of 15 µl using the Rotor-Gene SYBR® Green PCR Master Mix as described in Chialva et al. (2023).

Take-off and amplification efficiency values were calculated using the comparative quantitation mode in Rotor-Gene Q software and analysed using the Pfaffl (2001) equation using ubiquitin as reference gene (Fiorilli et al., 2009). The calibrated normalised relative quantities (CNRQ) of gene expression were calculated based on gene specific amplification efficiencies and normalised on the expression level of the reference gene. Oligonucleotides were designed for tomato transcripts (SL2.5 version from Sol Genomics database, http://solgenomics.net, Bombarely et al., 2011) using primer3 web software (http://bioinfo.ut.ee/primer3/) and purchased from Sigma-Aldrich (Supporting Information S1: Table S1).

2.6 | Donor-plant experiments

Donor-plant systems were set-up in 15-cm diameter round plastic pots sowing two *S. pennellii* plants at the centre of the pot and five peripheral plants being these *S. pennellii* itself (control), the domesticated tomato 'M82' and *Oryza sativa* 'IAC165' (strigolactone-overproducer, Jamil et al., 2012). Central and peripheral plants were separated by non-woven fabric to prevent interlace between central and peripheral root apparatus.

In a parallel experimental set-up, the same systems were established by separating inner and outer plants by a 50 µm or 0.5 µm mesh to allow or not, respectively, AMF hyphae to establish a mycorrhizal network. All these experiments were set-up under *F. mosseae* mycorrhizal inoculation (both outer and inner plants) as described above and watered twice a week with the modified Long-Ashton solution at 50 µM PO_4. For each condition three independent systems were set-up, randomising their position within the growth chamber each 2 days. After 60 days plants were sampled for AM colonisation analysis and a portion of roots stored at −80°C for molecular analyses.

2.7 | Treatments with GR24

S. pennellii plants were sown and potted as described above in 7 × 7 square plastic pots (100 mL) under *F. mosseae* inoculation. Plants

were treated with a synthetic analogue of strigolactones, GR24 (10 nM), or acetone as control which were supplemented to the watering solution (Long-Ashton, 50 µM P, twice a week) starting at 2 weeks from germination. For each condition five plants were grown and sampled after 60 days.

2.8 | Root exudate collection

S. pennellii and tomato 'M82' plants were grown in controlled conditions under different phosphate concentrations (500, 50, and 3 µM) using a modified Long-Ashton solution in a sterilised quartz-sand substrate. Plants were sampled after 40 days gently removing the substrate under tap water and root exudates were obtained from two plants under hydroponic conditions in 50 mL Falcon tubes containing 15 mL of sterile Long-Ashton solutions at the same phosphate level used during plant growth and under the same growth conditions to avoid stress responses. Three replicates for each condition were obtained. Root exudates were collected after 24 or 36 h, measuring the root fresh weight (RFW) from each Falcon tube (two plants) and diluting each exudate to the same ratio of root fresh weight per ml of exudate before performing *P. aegyptiaca* germination bioassays.

2.9 | *Phelipanche aegyptiaca* germination bioassays and solanacol quantification

To evaluate strigolactone synthesis and availability, germination bioassays of *P. aegyptiaca* seeds were performed as described previously (Prandi et al., 2011) with minor modifications. Seeds were surface-sterilised in 50% sodium hypochlorite in sterile water (10 min) and then rinsed three times with sterile dH_20 (10 min each). Twenty seeds were then placed under sterile conditions using a toothpick on a 6 cm diameter glass fibre disc placed over a two-layered filter paper disc (Whatman) moistened with 2 mL of sterile dH_20 and inserted in a 9 cm diameter Petri-dish. Petri-dishes were sealed with Parafilm® M and placed for 1 week at 26°C in the dark for seeds pre-conditioning. After 1 week, Petri-dishes were opened and allowed to dry out the residual moisture for at least 2 h under sterile conditions and treated with 2 mL of root exudates. GR24 and/or 5-Deoxystrigol (10^{-9} M) were used as positive control while water and Long-Ashton solutions as negative ones. For each of the three root exudate samples five technical replicates were set-up. Seeds were again incubated under the same conditions used for pre-conditioning for 1 week and germination scored under a stereo-microscope. Germination was considered successful if the radicle had protruded through the seed coat.

For solanacol quantification about 500 mg of fresh root material was extracted in 1 mL of ethyl acetate. Samples were dried in a Centrivap Vacuum system, resuspended in an acetonitrile/water mixture (67:33) and quantified in HPLC-MS/MS as described in Rial et al. (2019) using a Shimadzu Triple Quadrupole 8040 HPLC-MS system (Shimadzu) with an electrospray ionisation (ESI) source in positive ion mode, adding 33 µg L^{-1} of GR24 as internal standard. The chromatographic separation was achieved on a Kinetex C18 column (Phenomenex, 100 × 2.1 mm, 1.7 µm particle size, 100 Å) maintained at a constant temperature of 40°C. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in methanol), with a flow rate of 0.4 mL/min. A gradient elution was employed, starting from 50% B to 100% B over a 16-min period. The injection volume was set at 10 µL. The optimised MS parameters included a spray voltage of +5000 V, declustering potential of +100 V, entrance potential of +10 V, and a source temperature of 500°C. The Multiple Reaction Monitoring (MRM) mode was used to maximise signal intensity, focusing on the quantifier mass transition of 321–224 m/z.

2.10 | Statistical analyses and data visualisation

Statistical analyses were performed in the R statistical environment (R Core Team, 2023). Data normality and homoscedasticity were tested using Shapiro–Wilk (Shapiro & Wilk, 1965) and Levene's test (Levene, 1960) in the 'stats' and 'car' v3.1-2 packages (Fox & Weisberg, 2019), respectively ($p < 0.05$). According to data distributions, ANOVA for normal homoscedastic data or Kruskal–Wallis test for non-normal homoscedastic data (Kruskal & Wallis, 1952) were applied ($p < 0.05$) using custom base R function or the 'agricolae' package v1.3-7 (de Mendiburu, 2023). Multiple comparisons between treatments were performed using Tukey's HSD or Fisher's LSD tests after ANOVA or Kruskal-Wallis respectively, using the package 'agricolae' (both at $p < 0.05$). When only two experimental groups were compared the Student's *t*-test was applied as implemented in package 'ggpubr' v0.6.0 (Kassambara, 2023). All data visualisations were performed in R using 'ggplot2' v3.4.4 (Wickham, 2016).

3 | RESULTS AND DISCUSSION

3.1 | *S. lycopersicum* and its wild relatives differently respond to AM colonisation

To understand whether domestication has had an impact on the responsiveness and susceptibility of tomato to AMF, a first screening experiment was established under controlled conditions by using two tomato cultivars ('M82' and 'Moneymaker') and three wild relatives (*S. pennellii*, *S. neorickii* and *S. habrochaites*) encompassing the whole *Lycopersicon* clade (*Solanum* sect. *Lycopersicon*). All the plant genotypes were grown under *Funneliformis mosseae* inoculation (hereinafter MYC), an AMF known to efficiently colonise and promote the growth of tomato (Cesaro et al., 2020; Chialva et al., 2016) and compared to non-mycorrhizal controls (NM). Roots were sampled at a relatively late timepoint (60 days) allowing a steady AM colonisation. The morphological screening revealed that a

relatively high level of AM root colonisation is conserved within the *Lycopersicon* clade (median frequency (F%) > 60% in most of the accessions), with the exception of both *S. pennellii* accessions (CC2476, LA5240; Figure 1a). They showed a significantly reduced AM colonisation frequency (F%) and intensity (M%) compared to almost all the other genotypes. However, arbuscules abundance in the colonised portions of the root (a%) was similar (Figure 1a), suggesting that the capacity to support the full development of a colonisation unit (from the hyphopodium till the arbuscule) was maintained in all the genotypes.

Shoot fresh weight was negatively affected by mycorrhization in *S. pennellii* and *S. neorickii*, while the root fresh weight resulted lower in mycorrhizal plants of *S. pennellii* and in the domesticated tomato 'M82' compared to non-mycorrhizal plants (Supporting Information S1: Figure S1). SPAD values, which indicates the nutritional status, did not reveal a significant improvement under mycorrhizal conditions in nearly all the accessions tested. In the whole, the mycorrhizal growth response (MGR) resulted to be positive only for tomato 'Moneymaker' and *S. habrochaites*, while the *S. pennellii* accession LA5240 revealed the worst performance (Figure 1b).

According to a recent phylogeny reconstruction of *Lycopersicon* species (Li et al., 2023), *S. pennellii* results to be the farthest from cultivated tomatoes, having diverged from the common ancestor of the other wild and cultivated tomatoes around 1.97 Ma, together with *S. habrochaites*, while *S. neorickii* diverged more recently (around 1.73 Ma). However, irrespectively of the phylogenetic relationships, all the tested genotypes (with the exception of *S. pennellii*) revealed similar values of AM colonisation and MGR, indicating a limited beneficial effect by *F. mosseae* in both domesticated and wild relatives species. The data suggest that the AM parameters, here considered, are probably unrelated with tomato domestication events. This opens the question whether gene regulatory factors, plant physiology and environmental adaptations impact the mycorrhizal phenotype. Since *S. pennellii* (accession LA5240) revealed a clearcut negative response for the tested parameters (colonisation success, root and shoot development and MGR), further experiments were performed by comparing its responses with those of the tomato 'M82.'

3.2 | The low AM colonisation level of *S. pennellii* is independent of fungal identity and environmental conditions

To confirm the mycorrhizal phenotype observed in *S. pennellii*, the accession LA5240 was inoculated, in parallel to the cultivated tomato 'M82,' with a diverse fungal partner, *Rhizophagus irregularis*, which is considered an efficient generalist AMF. Since *S. pennellii* has been widely used in tomato breeding due to its drought tolerance (Kapazoglou

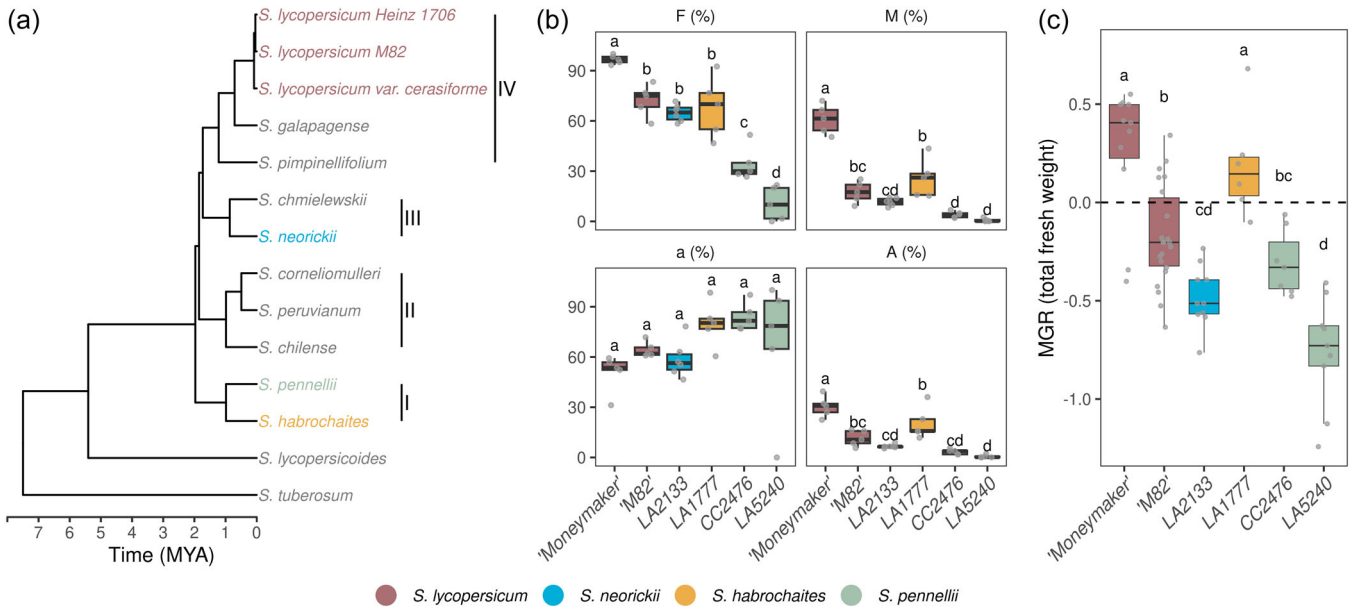

FIGURE 1 Mycorrhizal colonisation features in a selected panel of representative species within the *Solanum* sect. *Lycopersicon* clade inoculated with the arbuscular mycorrhizal fungus *Funneliformis mosseae* under controlled conditions and low phosphate availability (50 μM). (a) Dated phylogenomic tree of 12 representative species within the sect. *Lycoperiscon* modified from Li et al. (2023). The four clades within the phylogeny are indicated and *S. lycopersicoides* and *S. tuberosum* were used as outgroups. Wild-relative species investigated in this study are indicated with different colours. (b) Root colonisation parameters measured after cotton blue staining; F(%), frequency of mycorrhization, M(%) intensity of mycorrhization, a(%) arbuscules frequency in the colonised root segments, A(%) arbuscules frequency in the whole root apparatus of two *S. lycopersicum* cultivars ('Moneymaker' and 'M82'), *S. neorickii* LA2133, *S. habrochaites* LA1777 and two *S. pennellii* cultivars (CC2476, LA5240). (c) Mycorrhizal growth response (MGR) calculated on the total amount of fresh biomass. Box-plots display the median (horizontal line), the quartiles (boxes) and 1.5 interquartile range (whiskers). Letters indicate statistically supported differences according to Tukey's HSD post-hoc test after ANOVA ($p < 0.05$), $n = 5$. [Color figure can be viewed at wileyonlinelibrary.com]

et al., 2023), we also considered AM colonisation under combined water/nutrient stress applying a 35% reduction in nutrients and water provision (see methods for details). The combined stress had no effect on mycorrhization parameters of both genotypes (Figure 2a), with the exception of percentage of vesicles that were more abundant in the combined stress condition in tomato (Supporting Information S1: Figure S2). Notably, no vesicles were observed in *S. pennellii* We confirmed a low colonisation level in *S. pennellii* compared to tomato 'M82,' independently of AMF species used to inoculate plants (Supporting Information S1: Figure S3). We observed a significant reduction in frequency of mycorrhization (F%), intensity of root cortex mycorrhization (M%) and overall arbuscules abundance (A%) in *S. pennellii versus S. lycopersicum*. However, no differences were detected in arbuscules frequency (a%) and hypopodia density (Figure 2a). To check whether the limited colonisation of *S. pennellii* depends on defects in arbuscule development, arbuscule morphology was investigated using WGA staining and confocal imaging. Observations revealed regular branching and development of arbuscules in *S. pennelli*, similar to what was found in tomato 'M82' (Figure 2b,c).

To check whether arbuscule functioning was also maintained in *S. pennelli*, we checked the expression level of two plant genes considered as markers of nutrient exchanges occurring in arbusculated cells, *FatM* and *PT4*, which are involved in lipid metabolism and phosphate uptake (Lanfranco et al., 2018), respectively, as well as a fungal reference gene encoding for 18 S ribosomal RNA (van Tuinen et al., 1998), by using RT-qPCR. The expression of the three marker genes was upregulated upon AM colonisation (Supporting Information S1: Figure S4) irrespective of the lack of phosphate content increase in the shoot (Supporting Information S1: Figure S5). Similar expression values were recorded when *S. pennellii* was growing under stressed conditions (Supporting Information S1: Figure S4).

These data show that the low AM colonisation level of *S. pennellii* is independent of fungal identity and environmental conditions and suggest that the genetic background of *S. pennellii* is the main factor driving the limited extent of intraradical AMF colonisation. However, arbuscule morphology and the activation of plant genes involved in nutrient exchange witness the occurrence of a functional symbiosis.

3.3 | The *S. pennellii* mycorrhizal phenotype is heritable

To verify whether the hypothesis that *S. pennellii* has a limited susceptibility to AM symbiosis due to its genetic features, we tested

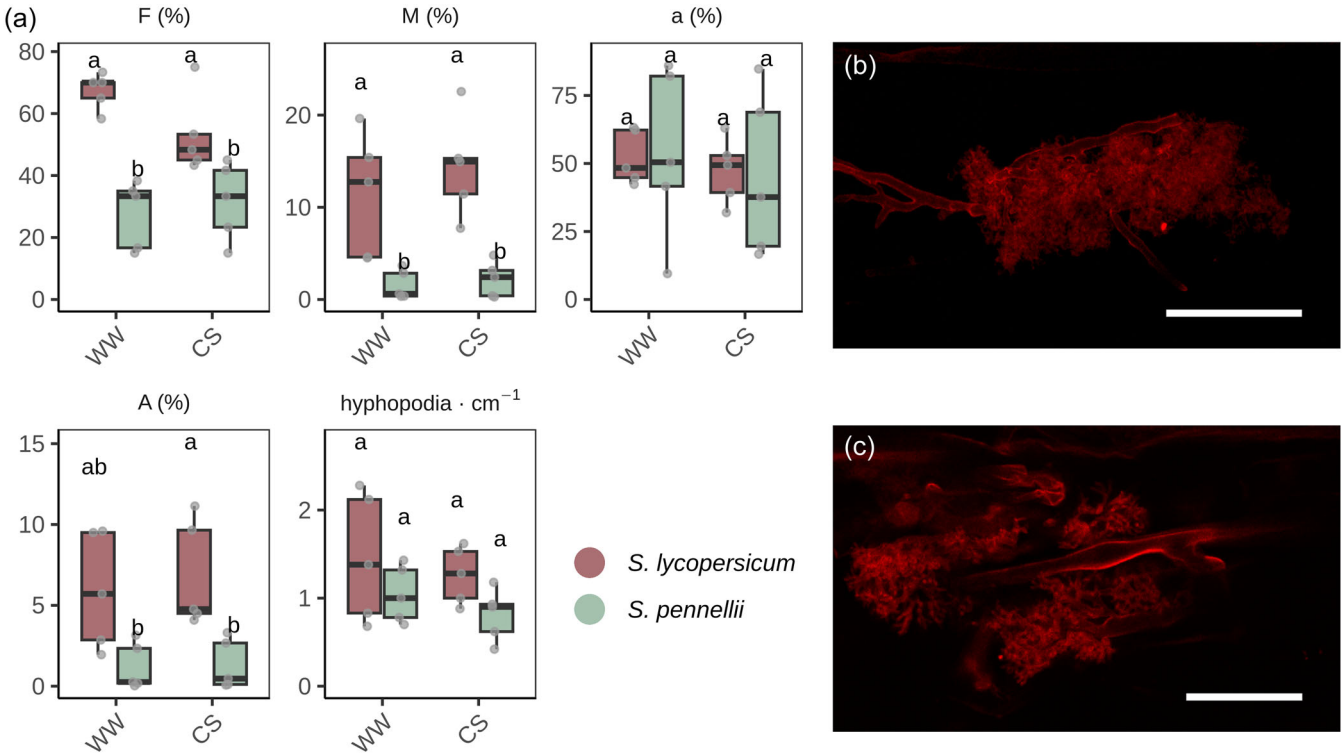

FIGURE 2 Mycorrhizal root colonisation *in S. pennellii* (LA5240) and *S. lycopersicum* 'M82' under combined water/nutrients stress (CS) and control conditions (WW) at 60 days after inoculation with the arbuscular mycorrhizal fungus *Funneliformis mosseae*. (a) Root colonisation parameters measured after cotton blue staining; F(%), frequency of mycorrhization, M(%) intensity of mycorrhization, a(%) arbuscules frequency in the colonised root segments, A(%) arbuscules frequency in the whole root apparatus. Box-plots display the median (horizontal line), the quartiles (boxes) and 1.5 interquartile range (whiskers). Letters indicate statistically supported differences according to Tukey's HSD post-hoc test after ANOVA ($p < 0.05$), $n = 5$. (b, c) WGA-FITC staining of arbuscules in *S. lycopersicum* (B) and *S. pennellii* (C) colonised roots; scale bar = 30 µm. [Color figure can be viewed at wileyonlinelibrary.com]

the AM susceptibility of a *S. lycopersicum* x *S. pennellii* F1 hybrid line (Torgeman & Zamir, 2023) at 60 days after inoculation with *F. mosseae*. The mycorrhization parameters analysed showed intermediate values between those registered for *S. lycopersicum* and *S. pennellii*, suggesting that the mycorrhizal traits of the parental lines are inherited (Supporting Information S1: Figure S6).

In addition, we searched the *S. pennellii* genome for presence of homologues of genes included in QTLs peaks already characterised as factors which regulate AM root colonisation in tomato (Plouznikoff et al., 2019). Fourty-two out of the 165 genes identified in the study showed no orthologs in *S. pennellii* (Supporting Information S1: Data S1). Interestingly, among the genes apparently lacking in the *S. pennellii* genome we found a high affinity sulphate transporter 1 (Solyc06g084140). These results support the hypothesis that the *S. pennellii* genetic background is the main factor responsible for the limited mycorrhization.

3.4 | Comparative transcriptomics reveals different patterns of responses to AM colonisation in *S. pennellii* and *S. lycopersicum*

To unravel molecular mechanisms involved in the regulation of the mycorrhizal phenotype observed in *S. pennellii*, we performed an RNA-seq experiment considering roots of *S. lycopersicum* 'M82' and *S. pennellii* (LA5240) plants grown under mycorrhizal colonisation by *F. mosseae* and non-inoculated treatment, under WW and CS conditions. The sequencing yielded ~15 to ~36 M quality-filtered read-pairs per library with a mean mapping rate of 82.9% and 76.7% on *S. lycopersicum* and *S. pennellii* transcriptome respectively (Supporting Information S1: Table S2). After the removal of low-expressed genes and normalisation, the data set resulted in 23,566 and 22,302 expressed genes in tomato and *S. pennellii* roots, which represents 65.9% and 45.9% of protein-coding genes (PCGs), respectively. Principal Component Analysis (PCA) showed a highly divergent pattern of gene expression upon mycorrhization in the two species (Figure 3a). Indeed, global variance partitioning analysis conducted on the whole normalised data sets highlighted a large contribution of AMF inoculation and both combined stress on transcriptome shifts, with similar proportions in the two species (Figure 3b). Notably, the figure of AM-regulated genes in *S. pennellii* was extremely low, not only in comparison with *S. lycopersicum* (545 vs 7317; Figure 3c), but also with other AM plants whose transcriptome has been investigated: differentially expressed genes (DEGs) in AM roots are usually more than one thousand, as observed in coffee (Chialva et al., 2023), *Lotus japonicus* (Venice et al., 2021) and rice (Hsieh et al., 2022). Mycorrhization in fact modulated 22.5% of the transcriptome in tomato (14.8% of PCGs) but only 2.5% in *S. pennellii* (1.1% PCGs). The ratio between up and down DEGs was similar in both species (Supporting Information S1: Figure S7).

To highlight differences between species in the transcriptional response to AM inoculation, differential expression analysis was performed considering the MYC vs NM contrast (Supporting Information S1: Data S2). DEGs were first validated using RT-qPCR assays on 7 *S. pennellii* selected genes among up- and down- regulated transcripts. RNA-seq and RT-qPCR results were consistent, showing a well supported correlation ($R^2 > 0.9$, $p < 0.05$; Supporting Information S1: Figure S8).

To further compare the differential expression profiles in tomato and *S. pennellii* we analysed orthologous gene pairs (Supporting Information S1: Figure S9) which were differentially regulated in MYC vs NM samples under both control and combined stress conditions and in both species (1822 genes; Figure 3d). The analysis revealed that the overlap of up- and down- regulated genes between the two species, considering the number of DEGs, was few (247) but consistent in terms of up- and downregulation. Interestingly, a highly diverged transcriptional landscape has been previously reported in *S. pennellii* when compared to other species within the clade (Koenig et al., 2013) also in response to environmental stimuli such as wounding (Liu et al., 2018).

Tomato 'M82' genotype reveals here a moderate responsiveness to AM symbiosis considering not only its growth response (Figure 1b), but also its induction of AM-related genes. Even if most of the nutrient transporters involved in AM interaction were upregulated (Supporting Information S1: Data S2), no functional categories related to nutrient acquisition emerged as enriched in DEGs under WW condition (Figure S10, Supporting Information S1: Data S3). By contrast, under the same condition and upon AM colonisation, *S. pennellii* showed a significant enrichment of functions related to nutrients transport and membrane remodelling, and decreased activation of genes involved in water transport and ROS metabolism. Among the most relevant categories dominated by upregulated genes (z-score > 0) 'transmembrane transport' (GO:0055085), 'plasma membrane' (GO:005886) and 'integral component of membrane' (GO:0016021) emerged. These regulations indicate that in *S. pennellii*, notwithstanding the AM-induced growth repression, some of the crucial symbiotic molecular responses are activated.

This transcriptional scenario deeply changed under combined stress conditions (CS): tomato revealed enriched categories with a dominance of upregulated genes, as the 'transmembrane transport' (GO:0055085), indicating that an improved nutrient acquisition occurred under AM-inoculation and stress conditions (Supporting Information S1: Figure S11). Differently, in *S. pennellii*, most of the categories previously dominated by upregulated genes under WW conditions were strongly downregulated. Among them, the membrane-related GO categories, which constitute a large portion of the downregulated genes (> 50% of DEGs in MYC vs NM contrast), emerged. This indicates that the increase of DEGs measured in MYC vs NM under CS condition in *S. pennellii* is mainly associated with the suppression of membrane-related mechanisms. On the other hand, the redox-related categories, which were downregulated under WW conditions, appeared among the upregulated categories (z-score > 0) in the CS conditions, accounting for a significant fraction of the overall DEGs number (~15%). Activation of redox metabolism is usually listed among the beneficial effects of AM symbiosis, particularly under stress conditions (Chandrasekaran et al., 2019). Interestingly, all these categories were downregulated in tomato, indicating a clear divergent response of the redox metabolism under combined stress between the two species.

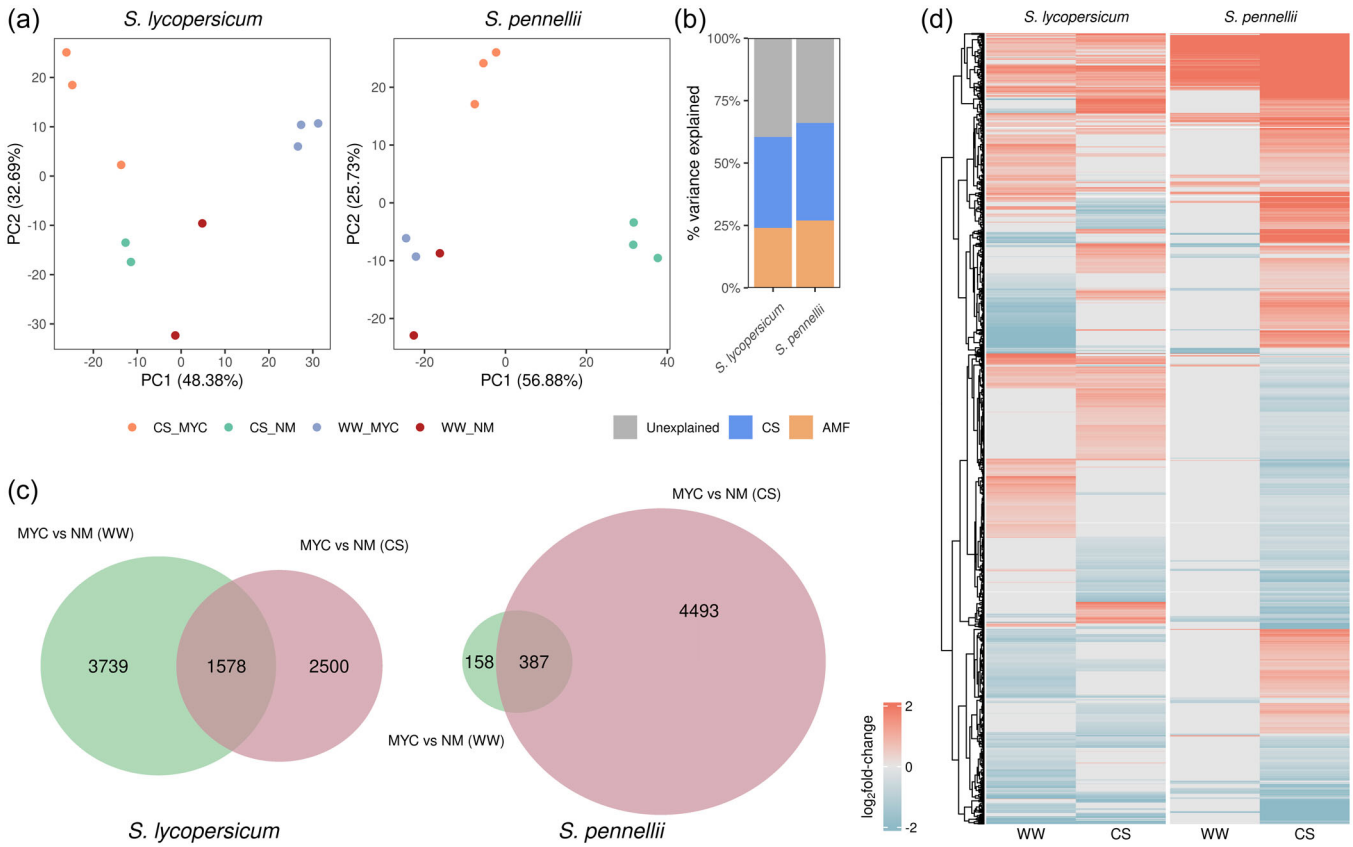

FIGURE 3 Comparative genome-wide root transcriptome analysis (RNA-seq) of *S. pennellii* (LA52140) and *S. lycopersicum* 'M82' at 60 days after inoculation with the arbuscular mycorrhizal fungus *Funneliformis mosseae* under combined water/nutrients stress (CS) and control well-watered conditions (WW). (a) Principal component analysis (PCA) and (b) variance partitioning analysis (VPA) of root transcriptomes under combined stress (CS) and mycorrhization (AMF). For each species, the amount of transcriptome variance explained by each of the experimental factors considered (mycorrhizal inoculation and combined stress) as well as the fraction not explained by any of the factors (unexplained) are shown. (c) Venn diagrams summarising the overlap between WW and CS conditions in AM-responsive (MYC vs NM) genes (FDR < 0.05) in tomato and *S. pennellii*. (d) Heatmap showing the extent of up- (red) and downregulation (blue) of AM-responsive orthologous genes in both species and conditions. [Color figure can be viewed at wileyonlinelibrary.com]

In conclusion, although important genes for AM symbiosis were activated in *S. pennellii* under WW condition, the number of DEGs was very limited compared to the extensive reprogramming normally observed in tomato (Zeng et al., 2023) as well as in other species (Handa et al., 2015); this could be due to low mycorrhization levels and could explain the lack of a positive effect on plant biomass. However, with similar mycorrhization levels, the transcriptional response was stronger in the combined stress condition. To better understand the reasons for such a peculiar behaviour, we looked in more detail at the expression pattern of crucial genes involved in symbiosis establishment and functioning.

3.5 | In *S. pennellii* AM relevant genes involved in CSSP and phosphate metabolism are regulated differently from tomato

We checked in our RNA-seq data set the expression pattern of 119 orthologous genes involved in AM signalling and functioning and in strigolactones and phosphate metabolism previously identified in legume and non-legumes species (Supporting Information S1: Data S4). Interestingly, most of the tomato genes showed a single orthologous gene in *S. pennellii* and almost all the genes involved in arbuscule functioning (Figure 4a), including *PT4*, *FatM*, *DIS*, *HA1* and *STR*, showed an upregulation in MYC versus NM plants in both species, also confirming the previous qPCR results (Supporting Information S1: Figure S3). Since we are comparing two different species and data obtained mapping sequences on two reference genomes (tomato and *S. pennellii*), we analysed the gene absolute expression value (expressed as TPM, transcripts per million reads). We found that in *S. pennellii* the basal expression of most of those genes under NM condition was lower than in tomato (Supporting Information S1: Figure S12). While we found a similar expression level of most of the genes involved in AM-related lipids metabolism and transfer (*FatM*, *DIS*, *STR*), a slightly decreased absolute expression was detected in *S. pennellii* for the AM-induced phosphate transporters PT4 and PT5 and for VAPYRIN (Supporting Information S1: Figure S12).

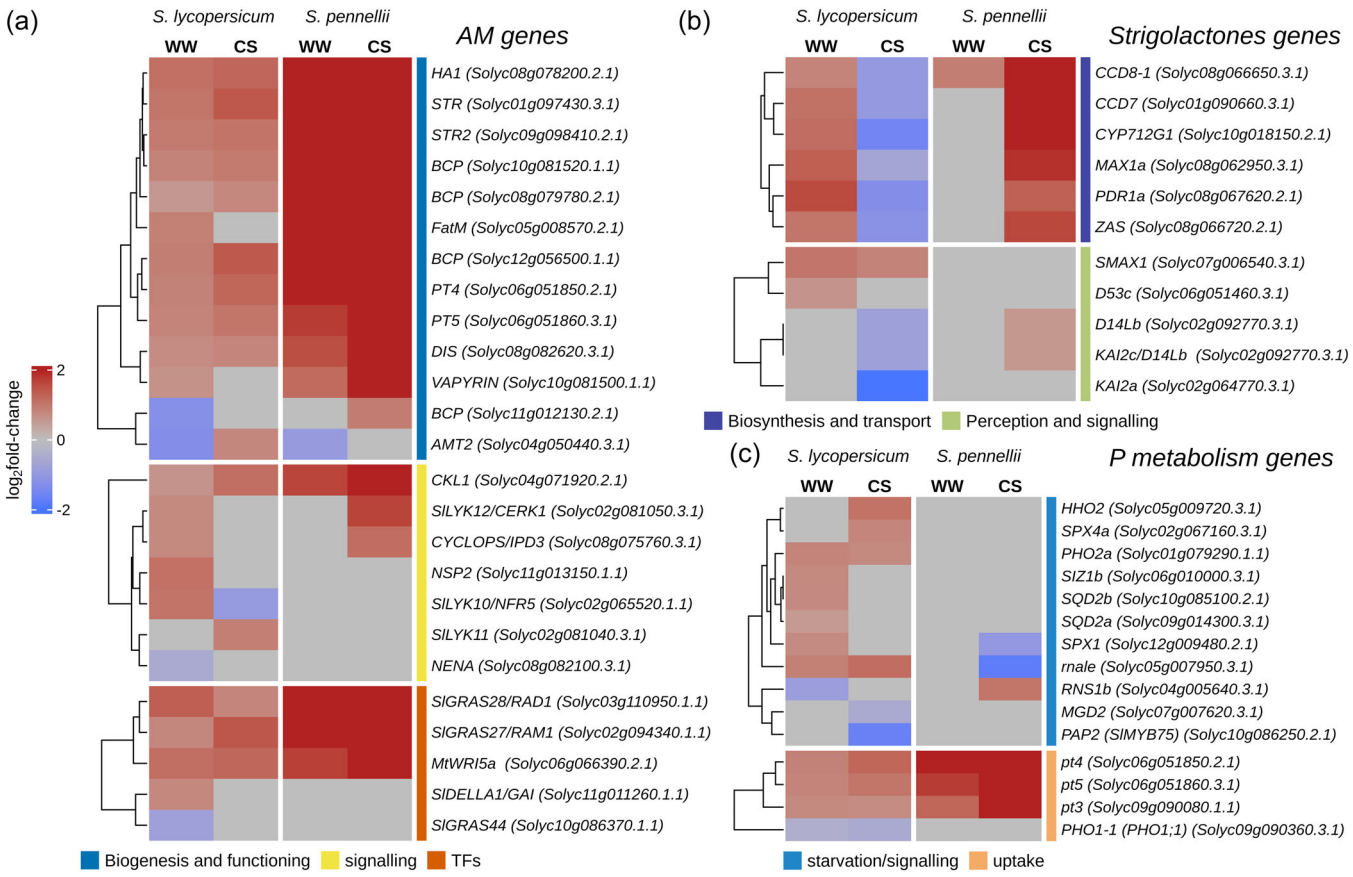

FIGURE 4 Differential expression (mycorrhizal *vs.* non-mycorrhizal comparison from RNA-seq experiment) of selected genes involved at different steps and processes of the mycorrhizal symbiosis in *S. lycopersicum* 'M82' and *S. pennellii* (accession LA5240) roots under control (WW) and combined water/nutrients stress conditions (CS). (a) Genes directly involved in different processes of the AM symbiosis, from signalling and accommodation to functioning. (b) Genes involved in strigolactones synthesis and perception. (c) Genes involved in phosphate starvation response and phosphorus assimilation. For each gene the symbol and the reference tomato transcript ID is reported in brackets. The manually-curated list of all the genes screened is provided in Supporting Information S1: Data S4. [Color figure can be viewed at wileyonlinelibrary.com]

PT4 and PT5 expression depends on PHR2, the transcription factor which is known to regulate the phosphate starvation response (PSR) and to control AM colonisation levels (Das et al., 2022; Shi et al., 2021). Indeed, PHR2 targets many AM-related genes through the binding of P1BS *cis-acting* element (GNATATNC) located on their promoters (Das et al., 2022). In tomato, at least three PHR2 orthologs have been identified, and one of them, SlPHL1, regulates PSR by directly upregulating the phosphate starvation induced genes via the P1BS motif (Zhang et al., 2021). In our data set, many genes involved in the PSR were modulated upon mycorrhization in tomato while none of them were AM-responsive in *S. pennellii* (Figure 4c), pointing to a differential regulation of the P metabolism in the two species. Interestingly, looking at *PT4* and *PT5* gene sequence, we found that in both promoters the P1BS motif is highly conserved, but - while in tomato it occurs three times within the first 4000 bp upstream of the CDS - it only occurs once in *S. pennellii PT4* and twice in *PT5* promoters (Supporting Information S1: Figure S13). We also looked at AW-box cis-elements, which occur in the promoters of many AM-related genes, including PT4 and STRs, and are recognized by WRI5a transcription factor, a master regulator of lipid provisioning and phosphate uptake pathway in mycorrhizal plants (Jiang et al., 2018; Zhang et al., 2023). While in the tomato PT4 promoter we found three AW-box-related motifs within 2 kb upstream of the coding sequence, in *S. pennellii* they occur at a higher distance from the ATG site (Supporting Information S1: Figure S13). This gene structure, with different number and distribution of AM-related cis-elements, could provide a mechanistic basis to explain a lower activation of AM-responsive genes in *S. pennellii* (Supporting Information S1: Figure S13).

Four gene orthologs involved in the common symbiotic signalling pathway (CSSP) displayed a lack of upregulation upon AM colonisation in *S. pennellii*, at least under optimal (well-watered) conditions (Figure 4). These genes include the *CYCLOPS/IPD3* and *NSP2* transcription factors (Ho-Plágaro & García-Garrido, 2022) and the lysin motif receptor-like kinase (LysM-RLKs) *SlLYK10*. Considering their absolute expression we found that these genes, along with others involved in AM signalling, were expressed with values similar to tomato. SlLYK10 has been identified as a putative myc-factor receptor in Solanaceae due to its high affinity for lipo-chito-oligosaccharides (Girardin et al., 2019) and its involvement in AM symbiosis establishment (Buendia et al., 2016). As already observed in these studies, *SlLYK10* is slightly upregulated under mycorrhizal

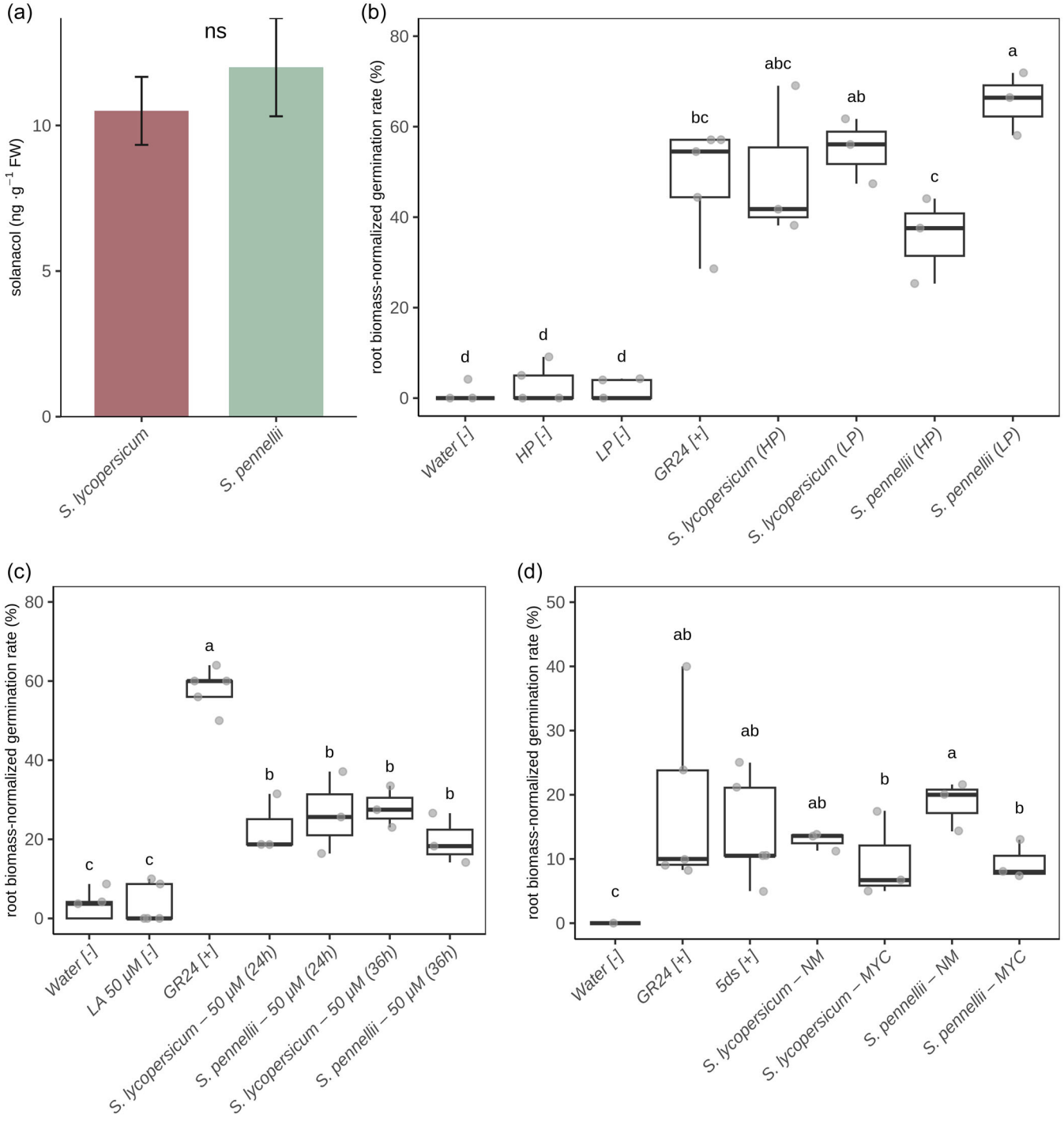

FIGURE 5 Measurements of roots and root exudates strigolactones content in *S. lycopersicum* 'M82' and *S. pennellii* (LA5240). (a) Solanacol content in *S. lycopersicum* and *S. pennellii* roots measured through HPLC-MS (see methods). Values are reported as ng g^{-1} of root fresh weight (FW); $n = 3$; no differences between the two species were found (Student's *t*-test, $p < 0.05$). (b) Germination of *Phelipanche aegyptiaca* seeds induced by root exudates of *S. pennellii* and *S. lycopersicum* obtained at high (HP, 500 µM) and low (LP, 3 µM) phosphate concentration after 24 h of exudation ($n = 3$). (c) Germination of *Phelipanche aegyptiaca* seeds induced by root exudates of *S. pennellii* and *S. lycopersicum* obtained at medium phosphate concentration (50 µM) after 24 and 36 h of exudation ($n = 3$). (d) Germination of *Phelipanche aegyptiaca* seeds induced by root exudates of *S. pennellii* (LA5240) and *S. lycopersicum* 'M82' obtained at medium phosphate concentration (50 µM) after 24 h of exudation, under mycorrhizal (MYC) and non-mycorrhizal (NM) conditions. In B, C and D water and/or Long-Ashton media in which root-exudates were collected were included as negative controls ($n = 5$), while positive controls were set-up using the synthetic strigolactones analogue GR24 and 5-Deoxystrigol (5DS); the percentage of germination was normalised on the total root biomass from which each extract was obtained; box-plots display the median (horizontal line), the quartiles (boxes) and 1.5 × interquartile range (whiskers); letters indicate statistically supported differences across genotypes according to the non-parametric pairwise Kruskall-Wallis test ($p < 0.05$). [Color figure can be viewed at wileyonlinelibrary.com]

colonisation in tomato under well-watered conditions. However, it is downregulated under combined stress conditions and not-regulated at all in *S. pennellii*. Notably, the *sllyk10* mutant in tomato displayed a significantly reduced root colonisation (Girardin et al., 2019), features which fully overlap with the *S. pennellii* mycorrhizal phenotype we observed here. Analysing the *SlLYK10* promoter, we found the highly conserved AAAGCTANNGACA *cis*-regulatory element (Supporting Information S1: Figure S13) which has been found as crucial for transcriptional regulation during AM symbiosis (Girardin et al., 2019), in both the species. However, while in the *SlLYK10* promoter of the tomato 'M82,' the P1BS motif is present at two sites, as in other RLKs receptors involved in AM symbiosis (i.e. OsCERK1; Das et al., 2022), in *S. pennellii* the P1BS motif occurs only once in *SlLYK10* ortholog and more than 1 kb upstream than in tomato. In addition, the comparative analysis of LYK10, revealed a conserved protein structure (Supporting Information S1: Figure S14); however, among 10 single amino acid mutations, one predicted as deleterious for protein function (EnsemblPlants variant vcZ13I2XU) only occurs in the *S. pennellii* sequence and not in other wild relative species.

Interestingly, among the already characterised LYK protein in tomato (Liao et al., 2018), we found the transcriptional induction upon mycorrhization of LYK12, one of the CERK1 paralogs in tomato, which however was differentially expressed under CS conditions but not under control conditions.

Taken in the whole, transcriptomic analyses reveal in *S. pennellii* the downregulation of genes belonging to the PSR network. This latter, with a cascade effect, has an impact on the expression of CSSP signalling genes. The ultimate reason might be associated with structural changes in the promoters of these genes.

3.6 | *S. pennellii* is competent for strigolactones production, but negatively modulates their biosynthesis under AM colonisation

We also looked at genes involved in metabolism and signalling of strigolactones (SLs) (Figure 4b) as they are modulators of root colonisation (Kobae et al., 2018; Lanfranco et al., 2018) also through the P starvation response (Das et al., 2022). Again in *S. pennellii* a number of these genes showed a contrasting expression pattern compared to tomato. Genes involved in SLs biosynthesis (*CCD7*, *CCD8* and *MAX1*), *PDR1*, a SLs exporter, and *ZAS*, which produces the apocarotenoid zaxinone that regulates SLs synthesis (Wang et al., 2019), were upregulated upon mycorrhization in tomato under well-watered conditions, while in *S. pennellii* they did not respond to the symbiosis. By contrast, under combined stress conditions all these genes were downregulated upon mycorrhization in tomato but upregulated in *S. pennellii* (Figure 4) while maintaining similar absolute expression levels in both species (Supporting Information S1: Figure S12).

To further investigate this issue, we analysed whether *S. pennellii* could produce and release SLs into root exudates. The amount of solanacol, the most abundant SLs in tomato, measured by HPLC-MS in root tissues, was similar in both species (Figure 5a). The amount of SLs in root exudates was also monitored using in vitro germination bioassays with seeds of the parasitic plant *Phelipanche aegyptiaca*. The experiments were performed on exudates obtained under different phosphate (Figure 5b) and timing conditions (Figure 5c). Results showed that *S. pennellii* exudates were always effective in stimulating parasitic seeds germination with few differences compared to tomato. While tomato did not respond to high (500 µM - HP) and low phosphate (3 µM—LP) levels (Figure 5b), *S. pennellii* significantly lowered the amount of SLs in the root exudates at HP compared to LP concentrations (Figure 5b), highlighting a different response to phosphate in the two species. Additionally, we analysed root exudates obtained from mycorrhizal plants and their non-mycorrhizal controls and observed that mycorrhization significantly reduced the amount of SLs exuded by *S. pennellii*, while no differences were observed in tomato (Figure 5d). Notably, we did not find in mycorrhizal *S. pennellii* plants under well-watered conditions the induction of the CYP712G1 gene (Solyc10g018150.2), a recently characterised gene responsible of solanacol biosynthesis (Wang et al., 2022). Interestingly, in our data set, the expression of this gene well-correlates with the induction/repression of the other SL biosynthesis genes in both the species. These data suggest that *S. pennellii* is fully competent for SLs production, but negatively modulates their biosynthesis under AM colonisation and P availability compared to tomato 'M82'. Previous studies showed that *S. pennellii* (accession LA0716) was less susceptible than cultivated tomato accessions to *P. aegyptiaca* infection (Bai et al., 2020; El-Halmouch et al., 2006) possibly due a reduced SLs exudation and SLs biosynthesis genes activation.

3.7 | Cocultivation with donor plants increases AM colonisation in *S. pennellii*

We hypothesised that an impaired amount of SLs released under MYC conditions may not be sufficient to promote the colonisation process, for example by stimulating new infection units. Plants were therefore treated with the synthetic SLs analogue GR24. Under that treatment root colonisation parameters displayed a slight trend to higher values compared to the untreated control (Supporting Information S1: Figure S15), which however were only statistically significant in the amount of arbuscules in mycorrhizal segments (a% parameter).

As a further experiment, we set-up a donor plant system in which *S. pennellii* plants were surrounded by the SLs-overproducer rice 'IAC165' (Jamil et al., 2012) with all plants inoculated with *F. mosseae* (Figure 6a). Compared to the control, in which the central *S. pennellii* plant was surrounded by other *S. pennellii* individuals, plants growing surrounded by the SLs-overproducer rice plants, showed enhanced root colonisation, reaching levels similar to those observed in tomato (Figure 6b), with consistent results across independent trials (Supporting Information S1: Figure S16). We found a significant increase in frequency (F%), intensity (M%) and overall abundance of

arbuscules in the root system (A%). This high level of AM colonisation was accompanied in the central *S. pennellii* plant by an increased expression of a number of AM-related genes, including *PT4*, *LYK10*, *CYCLOPS* and *NSP2*, while, by contrast *FatM* gene, involved in AM-dependent lipid transfer, was downregulated (Figure 6c).

Since these data suggested that the presence of donor plants, which continuously release SLs and likely provide nutrients to the fungus, enhanced mycorrhization in the central *S. pennellii* plants, we investigated whether this was due to SLs/diffusible molecules or C-derived nutrients.

In a new experiment, extra radical hyphae from donor plants (*Oryza sativa* 'IAC165', SLs-overproducer) were allowed to reach *S. pennellii* roots by crossing a mesh with 50 µm pores, or, alternatively, were blocked by a 0.5 µm pore mesh which only allowed the exudate flow. When the extraradical hyphae from rice donor plants could established a mycelial network reaching *S. pennellii* roots (50 µm pores), the colonisation was higher (Figure 7) compared to the plants where the presence of the 0.5 µm pores mesh, could only allow the movement of SLs or other diffusible molecules. It is worth to note that the *S. pennellii* biomass (shoot weight and length) was negatively affected by the higher colonisation level reached with the 50 µm pore membrane (Figure 7c). Differently from the previous donor experiment (Figure 6), here the expression level of *FatM* and *PT4* AM marker genes in both the conditions was similar (Supporting Information S1: Figure S17).

These results suggest that the connection to rice roots through a mycorrhizal network, and not the root exudates containing strigolactones, likely contributes to the increase in the AM colonisation level in *S. pennellii*. In addition, the lower biomass of *S. pennellii* plants showing higher colonisation levels indicates that the AM fungus is a cost for the plant. This was also suggested by the expression pattern of *FatM*, which was inversely proportional to the root colonisation levels. The lower expression level of this gene could be the strategy to avoid the expensive flow of reduced carbon to the AMF when an alternative C source is available (i.e. rice donors).

4 | CONCLUSION

Many strategies have been developed to identify favourable elite alleles and genes that can guarantee crop resilience and productivity under climate change conditions (Eckardt et al., 2023). Among them, the use of crop wild relatives as sources of superior traits for adaptation to abiotic and biotic stresses is very promising

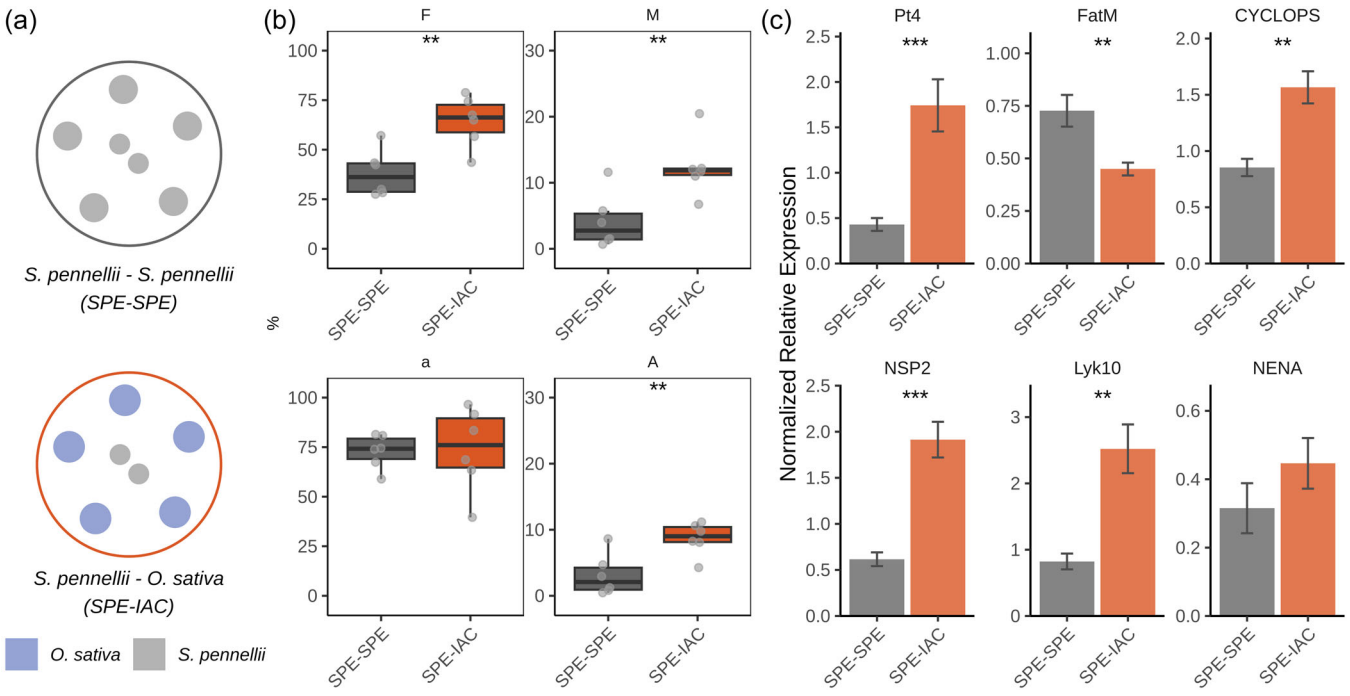

FIGURE 6 Main results from 'donor plant' experiments of *S. pennellii* (LA5240) collected 60 days after inoculation with the arbuscular mycorrhizal fungus *Funneliformis mosseae*. (a) Scheme summarising the experimental set-up in which *S. pennellii* plants (two plants at the centre of each pot, grey colour) were surrounded by five *O. sativa* 'IAC165' (SPE-IAC, blue colour) plants (strigolactones-overproducer) or by five *S. pennellii* plants as control (SPE-SPE). (b) Mycorrhizal colonisation parameters of central *S. pennellii* plants measured after cotton blue staining. F (%), frequency of mycorrhization, M(%) intensity of mycorrhization, a(%) arbuscules frequency in colonised roots, A(%) arbuscules frequency in the whole root apparatus. Box-plots display the median (horizontal line), the quartiles (boxes) and 1.5 interquartile range (whiskers); $n = 6$. (c) Expression level of five AM marker genes measured through RT-qPCR. Gene details and used primers are reported in Supporting Information S1: Table S1. Bar plots show the mean value ± SE ($n = 3$) of the relative expression level of each gene normalised on the expression of a reference Ubiquitin gene (Supporting Information S1: Table S1). In each plot, asterisks indicate statistically supported differences according to the Student's *t*-test (**, $p < 0.01$, ***, $p < 0.001$). [Color figure can be viewed at wileyonlinelibrary.com]

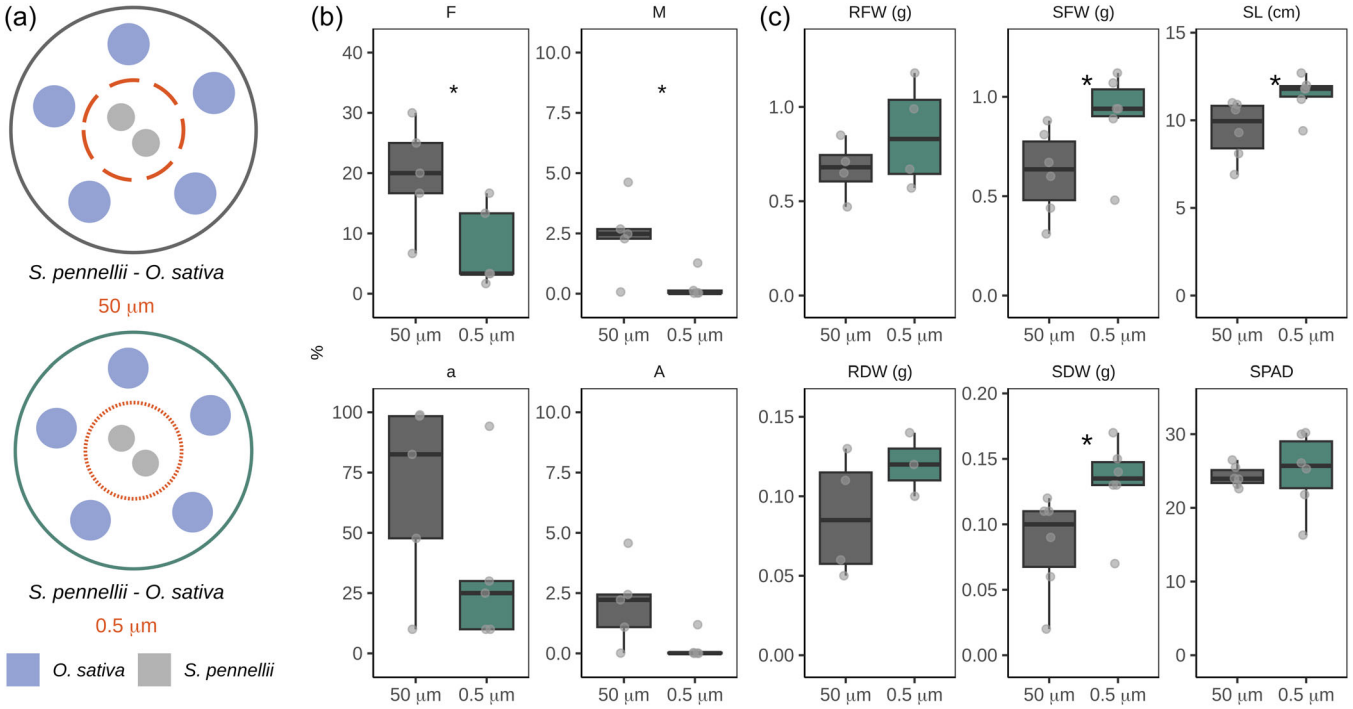

FIGURE 7 *S. pennellii* (LA5240) plants separated from the surrounding donor plants (strigolactone-overproducer *O. sativa* 'IAC165') by 0.5 µm or 50 µm mesh collected 60 days after inoculation with the arbuscular mycorrhizal fungus *Funneliformis mosseae*. (a) Scheme summarising the experimental set-up in which *S. pennellii* plants (two plants at the centre of each pot, grey colour) were surrounded by 5 *O. sativa* 'IAC165' (SPE-IAC, blue colour) plants (strigolactones-overproducer) separated with a 50 µm or a 0.5 µm pore mesh to allow or not the establishment of the mycorrhizal network. (b) Mycorrhizal colonisation parameters of central *S. pennellii* plants measured after cotton blue staining. F(%), frequency of mycorrhization, M(%) intensity of mycorrhization, a(%) arbuscules frequency in colonised roots, A(%) arbuscules frequency in the whole root apparatus; $n = 5$. (c) Growth parameter of the central *S. pennellii* plants; SL, shoot length, SFW, shoot fresh weight, RFW, root fresh weight, SPAD, SPAD index, TFW, total fresh weight; $n = 6$. Box-plots display the median (horizontal line), the quartiles (boxes) and 1.5 interquartile range (whiskers). Asterisks indicate statistically supported differences according to the Student's *t*-test (*, $p < 0.05$). [Color figure can be viewed at wileyonlinelibrary.com]

(Dempewolf et al., 2014). In this context, tomato wild relatives have been largely used to identify genes/alleles leading to superior traits. Among the many recent reports, Szymański et al. (2020) investigated backcrossed inbred lines which were developed using *S. pennellii* (LA0716) and *S. lycopersicum* 'M82'. They identified many genes and metabolites involved in pathogen defence and linked fungal resistance with changes in the fruit ripening regulatory network. However, the potential role of tomato wild relatives as a source of favourable traits for the accommodation of mycorrhizal fungi and of other beneficial components of the tomato microbiota and for systemic responses on the plant has been tested insofar only for recombinant inbred lines derived from *S. pimpinellifolium* (Plouznikoff et al., 2019), a wild tomato which diverged from *S. lycopersicum* in rather recent times (Li et al., 2023). In their genetic analysis Plouznikoff et al. (2019), by detecting AMF colonisation-related QTLs, demonstrated that wild alleles may improve AM symbiosis.

Here, we demonstrate that a panel of tomato wild relatives (the *S. pennellii* accession LA0716 and CC2476, *S. neorickii*, and *S. habrochaites*), all diverging from the today-cultivated tomatoes in very ancient times (from 7.5 to 1.7 Ma according to Li et al., 2023), is differently sensitive to the colonisation by AMF, when two parameters were considered, i.e. the extent of root colonisation and the mycorrhizal growth response (MGR). While *S. neorickii* and *S. habrochaites* revealed values largely comparable with those showed by *S. lycopersicum* 'M82' and 'Moneymaker' lines, the two S. *pennellii* accessions gave the worst performances in all the experiments, showing a very low colonisation level and a negative MGR. We can therefore conclude that, contrary to our hypothesis, the wild relative *S. pennellii* does not represent a source of useful alleles for promoting either root colonisation or the plant's AM-responsiveness.

The clear cut negative performance of *S. pennellii*, also after inoculation of the strong coloniser *R. irregularis*, under moderate water/nutrients stress conditions and with a very limited mineral nutrition (50 µM phosphate), opened the question to understand the molecular mechanisms behind such strong AM-symbiosis regulation. The genes which are required for the symbiosis, from the CSSP signalling genes to the transcription factors which activate the symbiotic functions of nutrient exchange, are all present in S. *pennellii* genome (Radhakrishnan et al., 2020). This is in line with the observation that, even if limited, the colonisation of *S. pennellii* roots shows normal arbuscule morphology and functioning. By contrast, some genes which have been identified within QTLs associated with

AMF colonisation in tomato (Plouznikoff et al., 2019), seem to be missing. Our transcriptomics data on mycorrhizal roots revealed a very low number of mycorrhiza-responsive genes (around 500) in *S. pennellii*, with an increase under combined stress conditions, indicating the interaction with environmental abiotic factors.

We demonstrate that in *S. pennellii* SLs synthesis is strongly sensitive not only to the fungal presence, but also to phosphate availability, differently from tomato 'M82.' Although lower SLs content in mycorrhizal plants may prevent secondary AMF infections, alteration in SLs metabolism is unlikely responsible for the limited AM susceptibility. A more probable explanation is that the fungus is perceived as a cost for the plant: our transcriptome data point to an inefficient activation of the metabolic pathways necessary to sustain the root-shoot axis that triggers plant growth. We hypothesise that the plant does not support a sufficient carbon transfer to the AMF; the donor plant experiments showed that the fungus can further proliferate in the root only when it is part of a mycorrhizal network, probably because it is fed by the surrounding rice plants. In this system a higher mycorrhization leads to an inhibition of plant growth, and this further limits the flow of lipids towards the fungus. Isotope-labelling experiments will be instrumental to verify this hypothesis.

The presence of genetic determinants that negatively impact the AM symbiosis in *S. pennellii* was confirmed from the analysis of the hybrid F1 line resulting from *S. pennellii* x *S. lycopersicum* 'M82' cross. The colonisation success was intermediate between that of the two parental lines, supporting the evidence that AMF colonisation level is a plant heritable trait. This also suggests that attention has to be given when S. *pennellii* introgression lines are developed, since AM negative traits can be moved to the new tomato varieties. However, only the characterization of back-cross lines will allow us to identify the responsible alleles (Torgeman and Zamir, 2023).

In conclusion, these unique features make *S. pennellii* an unprecedented model to study the molecular mechanisms which regulate the extent of AM colonisation in plant roots. It would be interesting to assess the mycotrophic status of this plant under natural conditions and verify whether it associates with peculiar AM fungi. We suggest that the use of tomato wild relatives as a source of new alleles requires deeper analysis which also considers the plant-beneficial microbe interaction.

ACKNOWLEDGEMENTS

This research has received funding from the European Union's Horizon 2020 research and innovation programme under grant no. 727929 (A novel and integrated approach to increase multiple and combined stress tolerance in plants using tomato as a model—TOMRES). The work was supported by the project CN_00000033 funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n. 3175 of 18 December 2021 of the Italian Ministry of University and Research funded by The European Union - NextGenerationEU. The authors thank Shai Torgeman and Dani Zamir (The Hebrew University of Jerusalem), Silvana Grandillo (National Research Council of Italy, Institute of Biosciences and Bioresources) and the Tomato Genetic Resource Center (TGRC, University of California Davis) for providing tomato and wild-relative seeds. The authors also thank Giorgio Buffa (University of Turin) for providing technical support in water potential measurements, Maria Teresa della Beffa (University of Turin) for plant care, Mara Politi, Yang Zhou, Gianluca Guazzotti, Francesca Lauria, Arianna Mulder, Ruben Chiaro and Giovanni Cultrera for lab assistance.

DATA AVAILABILITY STATEMENT

Raw sequencing data have been submitted to the Sequence Read Archive (SRA) repository (NCBI) under accession No. PRJNA1061177. Additional data related to the study is available at FigShare (https://doi.org/10.6084/m9.figshare.25213394).

ORCID

Matteo Chialva http://orcid.org/0000-0002-6996-6642
Stefania Stelluti https://orcid.org/0000-0003-3932-2675
Mara Novero https://orcid.org/0000-0001-7412-8750
Simon Masson http://orcid.org/0009-0000-2639-4130
Paola Bonfante https://orcid.org/0000-0003-3576-8530
Luisa Lanfranco https://orcid.org/0000-0002-3961-2552

REFERENCES

Ayankojo, I.T. & Morgan, K.T. (2020) Increasing air temperatures and its effects on growth and productivity of tomato in South Florida. *Plants*, 9(9), 1245. Available from: https://doi.org/10.3390/plants9091245

Bai, J., Wei, Q., Shu, J., Gan, Z., Li, B., Yan, D. et al. (2020) Exploration of resistance to *Phelipanche aegyptiaca* in tomato. *Pest Management Science*, 76(11), 3806–3821. Available from: https://doi.org/10.1002/ps.5932

Bolger, A., Scossa, F., Bolger, M.E., Lanz, C., Maumus, F., Tohge, T. et al. (2014) The genome of the stress-tolerant wild tomato species *Solanum pennellii*. *Nature Genetics*, 46(9), 1034–1038. Available from: https://doi.org/10.1038/ng.3046

Bombarely, A., Menda, N., Tecle, I.Y., Buels, R.M., Strickler, S., Fischer-York, T. et al. (2011) The sol genomics network (solgenomics.net): growing tomatoes using perl. *Nucleic Acids Research*, 39, D1149–D1155 Available from: https://doi.org/10.1093/nar/gkq866

Boufeldja, L., Brandt, D., Guzman, C., Vitou, M., Boudard, F., Morel, S. et al. (2022) Effect of elevated carbon dioxide exposure on nutrition-health properties of micro-Tom tomatoes. *Molecules*, 27(11), 3592. Available from: https://doi.org/10.3390/molecules27113592

Buendia, L., Wang, T., Girardin, A. & Lefebvre, B. (2016) The LysM receptor-like kinase SlLYK10 regulates the arbuscular mycorrhizal symbiosis in tomato. *New Phytologist*, 210(1), 184–195. Available from: https://doi.org/10.1111/nph.13753

Castañeda-Álvarez, N.P., Khoury, C.K., Achicanoy, H.A., Bernau, V., Dempewolf, H., Eastwood, R.J. et al. (2016) Global conservation priorities for crop wild relatives. *Nature Plants*, 2(4), 16022. Available from: https://doi.org/10.1038/nplants.2016.22

Celi, L., Cerli, C., Turner, B.L., Santoni, S. & Bonifacio, E. (2013) Biogeochemical cycling of soil phosphorus during natural revegetation of *Pinus sylvestris* on disused sand quarries in northwestern russia. *Plant and Soil*, 367(1–2), 121–134.

Cesaro, P., Massa, N., Cantamessa, S., Todeschini, V., Bona, E., Berta, G. et al. (2020) Tomato responses to funneliformis mosseae during the early stages of arbuscular mycorrhizal symbiosis. *Mycorrhiza*, 30(5), 601–610. Available from: https://doi.org/10.1007/s00572-020-00973-9

Chandrasekaran, M., Chanratana, M., Kim, K., Seshadri, S. & Sa, T. (2019) Impact of arbuscular mycorrhizal fungi on photosynthesis, water status, and gas exchange of plants under salt stress–a meta-analysis. *Frontiers in Plant Science*, 10, 457. Available from: https://doi.org/10.3389/fpls.2019.00457

Chialva, M., Lanfranco, L., Guazzotti, G., Santoro, V., Novero, M. & Bonfante, P. (2020) *Gigaspora margarita* and its endobacterium modulate symbiotic marker genes in tomato roots under combined water and nutrient stress. *Plants*, 9(7), 886. Available from: https://doi.org/10.3390/plants9070886

Chialva, M., Patono, D.L., de Souza, L.P., Novero, M., Vercellino, S., Maghrebi, M. et al. (2023) The mycorrhizal root-shoot axis elicits *Coffea arabica* growth under low phosphate conditions. *New Phytologist*, 239(1), 271–285. Available from: https://doi.org/10.1111/nph.18946

Chialva, M., Zouari, I., Salvioli, A., Novero, M., Vrebalov, J., Giovannoni, J.J. et al. (2016) Gr and hp-1 tomato mutants unveil unprecedented interactions between arbuscular mycorrhizal symbiosis and fruit ripening. *Planta*, 244(1), 155–165. Available from: https://doi.org/10.1007/s00425-016-2491-9

Das, D., Paries, M., Hobecker, K., Gigl, M., Dawid, C., Lam, H.-M. et al. (2022) PHOSPHATE STARVATION RESPONSE transcription factors enable arbuscular mycorrhiza symbiosis. *Nature Communications*, 13(1), 477. Available from: https://doi.org/10.1038/s41467-022-27976-8

Dempewolf, H., Eastwood, R.J., Guarino, L., Khoury, C.K., Müller, J.V. & Toll, J. (2014) Adapting agriculture to climate change: a global initiative to collect, conserve, and use crop wild relatives. *Agroecology and Sustainable Food Systems*, 38(4), 369–377. Available from: https://doi.org/10.1080/21683565.2013.870629

Diagne, N., Ngom, M., Djighaly, P.I., Fall, D., Hocher, V. & Svistoonoff, S. (2020) Roles of arbuscular mycorrhizal fungi on plant growth and performance: importance in biotic and abiotic stressed regulation. *Diversity*, 12(10), 370. Available from: https://doi.org/10.3390/d12100370

Dragićević, M.B., Paunović, D.M., Bogdanović, M.D., Todorović, S.I. & Simonović, A.D. (2020) ragp: pipeline for mining of plant hydroxyproline-rich glycoproteins with implementation in R. *Glycobiology*, 30(1), 19–35. Available from: https://doi.org/10.1093/glycob/cwz072

Eckardt, N.A., Cutler, S., Juenger, T.E., Marshall-Colon, A., Udvardi, M. & Verslues, P.E. (2023) Focus on climate change and plant abiotic stress biology. *The Plant Cell*, 35(1), 1–3. Available from: https://doi.org/10.1093/plcell/koac329

El-Halmouch, Y., Benharrat, H. & Thalouarn, P. (2006) Effect of root exudates from different tomato genotypes on broomrape (*O. aegyptiaca*) seed germination and tubercle development. *Crop Protection*, 25(5), 501–507. Available from: https://doi.org/10.1016/j.cropro.2005.08.005

Emms, D.M. & Kelly, S. (2019) OrthoFinder: phylogenetic orthology inference for comparative genomics. *Genome Biology*, 20(1), 238. Available from: https://doi.org/10.1186/s13059-019-1832-y

Fiorilli, V., Catoni, M., Miozzi, L., Novero, M., Accotto, G.P. & Lanfranco, L. (2009) Global and cell-type gene expression profiles in tomato plants colonized by an arbuscular mycorrhizal fungus. *New Phytologist*, 184(4), 975–987. Available from: https://doi.org/10.1111/j.1469-8137.2009.03031.x

Fox, J. & Weisberg, S. (2019) *An R Companion to Applied Regression*, 3rd edition, Thousand Oaks, CA, USA: Sage, p. 608. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Fracasso, A., Telò, L., Lanfranco, L., Bonfante, P. & Amaducci, S. (2020) Physiological beneficial effect of *Rhizophagus intraradices* inoculation on tomato plant yield under water deficit conditions. *Agronomy*, 10(1), 71. Available from: https://doi.org/10.3390/agronomy10010071

de Freitas, S.T., Shackel, K.A. & Mitcham, E.J. (2011) Abscisic acid triggers whole-plant and fruit-specific mechanisms to increase fruit calcium uptake and prevent blossom end rot development in tomato fruit. *Journal of Experimental Botany*, 62(8), 2645–2656. Available from: https://doi.org/10.1093/jxb/erq430

Genre, A., Lanfranco, L., Perotto, S. & Bonfante, P. (2020) Unique and common traits in mycorrhizal symbioses. *Nature Reviews Microbiology*, 18(11), 649–660. Available from: https://doi.org/10.1038/s41579-020-0402-3

Girardin, A., Wang, T., Ding, Y., Keller, J., Buendia, L., Gaston, M. et al. (2019) LCO receptors involved in arbuscular mycorrhiza are functional for rhizobia perception in legumes. *Current Biology*, 29(24), 4249–4259.e5. Available from: https://doi.org/10.1016/j.cub.2019.11.038

Handa, Y., Nishide, H., Takeda, N., Suzuki, Y., Kawaguchi, M. & Saito, K. (2015) RNA-seq transcriptional profiling of an arbuscular mycorrhiza provides insights into regulated and coordinated gene expression in *Lotus japonicus* and *Rhizophagus irregularis*. *Plant and Cell Physiology*, 56(8), 1490–1511. Available from: https://doi.org/10.1093/pcp/pcv071

Hoeksema, J.D., Chaudhary, V.B., Gehring, C.A., Johnson, N.C., Karst, J., Koide, R.T. et al. (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecology Letters*, 13(3), 394–407. Available from: https://doi.org/10.1111/j.1461-0248.2009.01430.x

Ho-Plágaro, T. & García-Garrido, J.M. (2022) Multifarious and interactive roles of GRAS transcription factors during arbuscular mycorrhiza development. *Frontiers in Plant Science*, 13, 836213. Available from: https://doi.org/10.3389/fpls.2022.836213

Hsieh, C., Chen, Y.-H., Chang, K.-C. & Yang, S.-Y. (2022) Transcriptome analysis reveals the mechanisms for mycorrhiza-enhanced salt tolerance in rice. *Frontiers in Plant Science*, 13, 1072171. https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2022.1072171

Hu, B., Jin, J., Guo, A.-Y., Zhang, H., Luo, J. & Gao, G. (2015) GSDS 2.0: an upgraded gene feature visualization server. *Bioinformatics*, 31(8), 1296–1297. Available from: https://doi.org/10.1093/bioinformatics/btu817

Jamil, M., Charnikhova, T., Houshyani, B., van Ast, A. & Bouwmeester, H.J. (2012) Genetic variation in strigolactone production and tillering in rice and its effect on *Striga hermonthica* infection. *Planta*, 235(3), 473–484. Available from: https://doi.org/10.1007/s00425-011-1520-y

Jiang, Y., Xie, Q., Wang, W., Yang, J., Zhang, X., Yu, N. et al. (2018) Medicago AP2-domain transcription factor WRI5a is a master regulator of lipid biosynthesis and transfer during mycorrhizal symbiosis. *Molecular Plant*, 11(11), 1344–1359Available from: https://doi.org/10.1016/j.molp.2018.09.006

Johnson, N.C., Wilson, G.W.T., Wilson, J.A., Miller, R.M. & Bowker, M.A. (2015) Mycorrhizal phenotypes and the law of the minimum. *New Phytologist*, 205(4), 1473–1484. Available from: https://doi.org/10.1111/nph.13172

Kapazoglou, A., Gerakari, M., Lazaridi, E., Kleftogianni, K., Sarri, E., Tani, E. et al. (2023) Crop wild relatives: a valuable source of tolerance to various abiotic stresses. *Plants*, 12(2), 328. Available from: https://doi.org/10.3390/plants12020328

Kassambara, A. (2023) *ggpubr: 'ggplot2' Based Publication Ready Plots*. https://CRAN.R-project.org/package=ggpubr

Kobae, Y., Kameoka, H., Sugimura, Y., Saito, K., Ohtomo, R., Fujiwara, T. et al. (2018) Strigolactone biosynthesis genes of rice are required for the punctual entry of arbuscular mycorrhizal fungi into the roots. *Plant and Cell Physiology*, 59(3), 544–553. Available from: https://doi.org/10.1093/pcp/pcy001

Koenig, D., Jiménez-Gómez, J.M., Kimura, S., Fulop, D. & Chitwood, D.H. (2013) Comparative transcriptomics reveals patterns of selection in

domesticated and wild tomato. *Proceedings of the National Academy of Sciences*, 110(28), E2655–E2662. https://doi.org/10.1073/pnas.1309606110

Kruskal, W.H. & Wallis, W.A. (1952) Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association*, 47(260), 583–621Available from: https://doi.org/10.1080/01621459.1952.10483441

Lanfranco, L., Fiorilli, V. & Gutjahr, C. (2018) Partner communication and role of nutrients in the arbuscular mycorrhizal symbiosis. *New Phytologist*, 220(4), 1031–1046. Available from: https://doi.org/10.1111/nph.15230

Leek, J.T., Johnson, W.E., Parker, H.S., Fertig, E.J., Jaffe, A.E., Zhang, Y. et al. (2023) *sva: Surrogate Variable Analysis*. R package version 3.32.1. https://doi.org/10.18129/B9.bioc.sva

Lehnert, H., Serfling, A., Enders, M., Friedt, W. & Ordon, F. (2017) Genetics of mycorrhizal symbiosis in winter wheat (*Triticum aestivum*). *New Phytologist*, 215(2), 779–791. Available from: https://doi.org/10.1111/nph.14595

Levene, H. (1960) Robust tests for equality of variances, In:. *Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling*. Palo Alto, CA, I. Olkin: Stanford University Press. pp. 278–292

Leventis, G., Tsiknia, M., Feka, M., Ladikou, E.V., Papadakis, I.E., Chatzipavlidis, I. et al. (2021) Arbuscular mycorrhizal fungi enhance growth of tomato under normal and drought conditions, *via* different water regulation mechanisms. *Rhizosphere*, 19, 100394. Available from: https://doi.org/10.1016/j.rhisph.2021.100394

Li, J., Scarano, A., Gonzalez, N.M., D'Orso, F., Yue, Y., Nemeth, K. et al. (2022) Biofortified tomatoes provide a new route to vitamin D sufficiency. *Nature Plants*, 8(6), 611–616. Available from: https://doi.org/10.1038/s41477-022-01154-6

Li, N., He, Q., Wang, J., Wang, B., Zhao, J., Huang, S. et al. (2023) Super-pangenome analyses highlight genomic diversity and structural variation across wild and cultivated tomato species. *Nature Genetics*, 55(5), 852–860. Available from: https://doi.org/10.1038/s41588-023-01340-y

Liao, D., Sun, X., Wang, N., Song, F. & Liang, Y. (2018) Tomato LysM receptor-like kinase SlLYK12 is involved in arbuscular mycorrhizal symbiosis. *Frontiers in Plant Science*, 9, 346749. Available from: https://doi.org/10.3389/fpls.2018.01004

Liu, M.-J., Sugimoto, K., Uygun, S., Panchy, N., Campbell, M.S., Yandell, M. et al. (2018) Regulatory divergence in Wound-Responsive gene expression between domesticated and wild tomato. *The Plant Cell*, 30(7), 1445–1460. Available from: https://doi.org/10.1105/tpc.18.00194

Love, M.I., Huber, W. & Anders, S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq. 2. *Genome Biology*, 15(12), 550. Available from: https://doi.org/10.1186/s13059-014-0550-8

Martín-Robles, N., Lehmann, A., Seco, E., Aroca, R., Rillig, M.C. & Milla, R. (2018) Impacts of domestication on the arbuscular mycorrhizal symbiosis of 27 crop species. *New Phytologist*, 218(1), 322–334. Available from: https://doi.org/10.1111/nph.14962

de Mendiburu, F. (2023) *agricolae: Statistical Procedures for Agricultural Research*. R package version 1.3-7. https://CRAN.R-project.org/package=agricolae.

Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P. et al. (2022) *vegan: Community Ecology Package*. R package v2.6-4. https://CRAN.R-project.org/package=vegan.

Patro, R., Duggal, G., Love, M.I., Irizarry, R.A. & Kingsford, C. (2017) Salmon provides fast and bias-aware quantification of transcript expression. *Nature Methods*, 14(4), 417–419. Available from: https://doi.org/10.1038/nmeth.4197

Pessoa, H.P., Dariva, F.D., Copati, M.G.F., de Paula, R.G., de Oliveira Dias, F. & Gomes, C.N. (2023) Uncovering tomato candidate genes associated with drought tolerance using *Solanum pennellii* introgression lines. *PLoS One*, 18(6), e0287178. Available from: https://doi.org/10.1371/journal.pone.0287178

Pfaffl, M.W. (2001) A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Research*, 29(9), 45e.

Plouznikoff, K., Asins, M.J., de Boulois, H.D., Carbonell, E.A. & Declerck, S. (2019) Genetic analysis of tomato root colonization by arbuscular mycorrhizal fungi. *Annals of Botany*, 124(6), 933–946. Available from: https://doi.org/10.1093/aob/mcy240

Prandi, C., Occhiato, E.G., Tabasso, S., Bonfante, P., Novero, M., Scarpi, D. et al. (2011) New potent fluorescent analogues of strigolactones: synthesis and biological activity in parasitic weed germination and fungal branching. *European Journal of Organic Chemistry*, 2011(20–21), 3781–3793. Available from: https://doi.org/10.1002/ejoc.201100616

R Core Team (2023) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/

Radhakrishnan, G.V., Keller, J., Rich, M.K., Vernié, T., Mbadinga Mbadinga, D.L., Vigneron, N. et al. (2020) An ancestral signalling pathway is conserved in intracellular symbioses-forming plant lineages. *Nature Plants*, 6(3), 280–289. Available from: https://doi.org/10.1038/s41477-020-0613-7

Rau, A., Gallopin, M., Celeux, G. & Jaffrézic, F. (2013) Data-based filtering for replicated high-throughput transcriptome sequencing experiments. *Bioinformatics*, 29(17), 2146–2152. Available from: https://doi.org/10.1093/bioinformatics/btt350

Rial, C., Varela, R.M., Molinillo, J.M.G., López-Ráez, J.A. & Macías, F.A. (2019) A new UHPLC-MS/MS method for the direct determination of strigolactones in root exudates and extracts. *Phytochemical Analysis*, 30(1), 110–116. Available from: https://doi.org/10.1002/pca.2796

Risso, D., Ngai, J., Speed, T.P. & Dudoit, S. (2014) Normalization of RNA-seq data using factor analysis of control genes or samples. *Nature Biotechnology*, 32(9), 896–902. Available from: https://doi.org/10.1038/nbt.2931

Saadi, S., Todorovic, M., Tanasijevic, L., Pereira, L.S., Pizzbigalli, C. & Lionello, P. (2015) Climate change and Mediterranean agriculture: impacts on winter wheat and tomato crop evapotranspiration, irrigation requirements and yield. *Agricultural Water Management*, 147, 103–115. Available from: https://doi.org/10.1016/j.agwat.2014.05.008

Sato, S., Tabata, S., Hirakawa, H., Asamizu, E., Shirasawa, K., Sachiko, I, et al. (2012) The tomato genome sequence provides insights into fleshy fruit evolution. *Nature*, 485(7400), 635–641. Available from: https://doi.org/10.1038/nature11119

Shapiro, S.S. & Wilk, M.B. (1965) An analysis of variance test for normality (complete samples). *Biometrika*, 52(3–4), 591–611. Available from: https://doi.org/10.1093/biomet/52.3-4.591

Shi, J., Zhao, B., Zheng, S., Zhang, X., Wang, X., Dong, W. et al. (2021) A phosphate starvation response-centered network regulates mycorrhizal symbiosis. *Cell*, 184(22), 5527–5540.e18. Available from: https://doi.org/10.1016/j.cell.2021.09.030

Smith, S.E., Smith, F.A. & Jakobsen, I. (2003) Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology*, 133(1), 16–20. Available from: https://doi.org/10.1104/pp.103.024380

Soneson, C., Love, M.I. & Robinson, M.D. (2015) Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. *F1000Research*, 4, 1521. Available from: https://doi.org/10.12688/f1000research.7563.1

Szymański, J., Bocobza, S., Panda, S., Sonawane, P., Cárdenas, P.D., Lashbrooke, J. et al. (2020) Analysis of wild tomato introgression lines elucidates the genetic basis of transcriptome and metabolome variation underlying fruit traits and pathogen response. *Nature Genetics*, 52(10), 1111–1121. Available from: https://doi.org/10.1038/s41588-020-0690-6

Torgeman, S. & Zamir, D. (2023) Epistatic QTLs for yield heterosis in tomato. *Proceedings of the National Academy of Sciences*, 120(14), e2205787119. https://doi.org/10.1073/pnas.2205787119

Trouvelot, A., Kough, J. & Gianinazzi-Pearson, V. (1986) Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. In: Gianinazzi, S. & Gianinazzi-Pearson, V. (eds.) *Physiological and Genetical Aspects of Mycorrhizae*. INRA Press. pp. 217–221.

van Tuinen, D., Jacquot, E., Zhao, B., Gollotte, A. & Gianinazzi-Pearson, V. (1998) Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. *Molecular Ecology*, 7(7), 879–887. Available from: https://doi.org/10.1046/j.1365-294x.1998.00410.x

Ullah, F., Ullah, H., Ishfaq, M., Gul, S.L., Kumar, T. & Li, Z. (2023) Improvement of nutritional quality of tomato fruit with funneliformis mosseae inoculation under greenhouse conditions. *Horticulturae*, 9(4), 448. Available from: https://doi.org/10.3390/horticulturae9040448

Venice, F., Chialva, M., Domingo, G., Novero, M., Carpentieri, A., Salvioli di Fossalunga, A. et al. (2021) Symbiotic responses of *Lotus japonicus* to two isogenic lines of a mycorrhizal fungus differing in the presence/absence of an endobacterium. *The Plant Journal*, 108(6), 1547–1564. Available from: https://doi.org/10.1111/tpj.15578

Walter, W., Sánchez-Cabo, F. & Ricote, M. (2015) GOplot: an R package for visually combining expression data with functional analysis. *Bioinformatics*, 31(17), 2912–2914.

Wang, J.Y., Haider, I., Jamil, M., Fiorilli, V., Saito, Y., Mi, J. et al. (2019) The apocarotenoid metabolite zaxinone regulates growth and strigolactone biosynthesis in rice. *Nature Communications*, 10(1), 810. Available from: https://doi.org/10.1038/s41467-019-08461-1

Wang, Y., Durairaj, J., Suárez Duran, H.G., van Velzen, R., Flokova, K., Liao, C.-Y. et al. (2022) The tomato cytochrome P450 CYP712G1 catalyses the double oxidation of orobanchol en route to the rhizosphere signalling strigolactone, solanacol. *New Phytologist*, 235(5), 1884–1899. Available from: https://doi.org/10.1111/nph.18272

Wickham, H. (2016) *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org

Young, M.D., Wakefield, M.J., Smyth, G.K. & Oshlack, A. (2010) Gene ontology analysis for RNA-seq: accounting for selection bias. *Genome Biology*, 11(2), R14. Available from: https://doi.org/10.1186/gb-2010-11-2-r14

Zeng, Z., Liu, Y., Feng, X.-Y., Li, S.-X., Jiang, X.-M., Chen, J.-Q. et al. (2023) The RNAome landscape of tomato during arbuscular mycorrhizal symbiosis reveals an evolving RNA layer symbiotic regulatory network. *Plant Communications*, 4(1), 100429. Available from: https://doi.org/10.1016/j.xplc.2022.100429

Zhang, Q., Wang, S., Xie, Q., Xia, Y., Lu, L., Wang, M. et al. (2023) Control of arbuscule development by a transcriptional negative feedback loop in Medicago. *Nature Communications*, 14(1), 5743. Available from: https://doi.org/10.1038/s41467-023-41493-2

Zhang, Y., Wang, Y., Wang, E., Wu, X., Zheng, Q., Han, Y. et al. (2021) SlPHL1, a MYB-CC transcription factor identified from tomato, positively regulates the phosphate starvation response. *Physiologia Plantarum*, 173(3), 1063–1077. Available from: https://doi.org/10.1111/ppl.13503

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Chialva, M., Stelluti, S., Novero, M., Masson, S., Bonfante, P. & Lanfranco, L. (2024) Genetic and functional traits limit the success of colonisation by arbuscular mycorrhizal fungi in a tomato wild relative. *Plant, Cell & Environment*, 1–18. https://doi.org/10.1111/pce.15007