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Received: 13 July 2022 | Revised: 4 January 2023 | Accepted: 11 January 2023

DOI: 10.1111/ppl.13857

ORIGINAL RESEARCH

Physiologia Plantarum

Carbohydrate and lipid balances in the positive plant phenotypic response to arbuscular mycorrhiza: increase in sink strength

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Funding information
Consejo Nacional de Ciencia y Tecnología, Grant/Award Number: 732373; Coordinación de la Investigación Científica, Grant/Award Number: 5872608

Edited by H. Saitoh

Abstract

The exchange of phosphorus (P) and carbon (C) between plants and arbuscular mycorrhizal fungi (AMF) is a major determinant of their mutualistic symbiosis. We explored the C dynamics in tomato (*Solanum lycorpersicum*) inoculated or not with *Rhizophagus irregularis* to study their growth response under different NaH_2PO_4 concentrations (Null P, 0 mM; Low P, 0.065 mM; High P, 1.3 mM). The percentage of AMF colonization was similar in plants under Null and Low P, but severely reduced under High P. However, the AMF mass biomarker 16:1ω5 revealed higher fungal accumulation in inoculated roots under Low P, while more AMF spores were produced in the Null P. Under High P, AMF biomass and spores were strongly reduced. Plant growth response to mycorrhiza was negative under Null P, showing reduction in height, biovolume index, and source leaf (SL) area. Under Low P, inoculated plants showed a positive response (e.g., increased SL area), while inoculated plants under High P were similar to non-inoculated plants. AMF promoted the accumulation of soluble sugars in the SL under all fertilization levels, whereas the soluble sugar level decreased in roots under Low P in inoculated plants. Transcriptional upregulation of *SlLIN6* and *SlSUS1*, genes related to carbohydrate metabolism, was observed in inoculated roots under Null P and Low P, respectively. We conclude that P-limiting conditions that increase AMF colonization stimulate plant growth due to an increase in the source and sink strength. Our results suggest that C partitioning and allocation to different catabolic pathways in the host are influenced by AMF performance.

1 | INTRODUCTION

Carbon dioxide is the main source of carbon (C) for plants. The photosynthetic leaves of vascular plants, aka source tissues, use C to produce sucrose (Suc) and starch as the end-products of photosynthesis. Suc is the main soluble carbohydrate that most plants use to translocate C from source to non-photosynthetic or sink tissues. The partitioning and allocation of C in plants are mediated by a complex molecular machinery involving processes related to cell differentiation and organ development during the plant ontogenetic cycle (Poorter & Nagel, 2000; Poorter & Pothmann, 1992). During these processes, the activities of key enzymes involved in Suc biosynthesis, such as Suc-P

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synthase (SPS; EC 2.4.1.14) and fructose bisphosphatase (EC 3.1.3.11) (Chen et al., 2005; Kerr & Huber, 1987; Lunn & ap Rees, 1990), as well as the Suc catabolic enzymes Suc synthase (SuSy; EC 2.4.1.13) and invertase (EC 3.2.1.26), greatly influence the final outcome (Bihmidine et al., 2013; Koch, 2004; Koch et al., 1996). The dynamics of C in plants are also determined by the activity of transport proteins, such as the monosaccharide transporters from the major facilitator superfamilies: SUGAR WILL EVENTUALLY BE EXPORTED TRANSPORTER (SWEET) and SUC TRANSPORTER (SUT) (Braun, 2012; Kühn & Grof, 2010; Reuscher et al., 2014).

Plants can establish complex mutualistic relationships with microorganisms. The complexity of these relationships lies in the fact that they have the potential to modify the activities of source or sink tissues or both, altering the dynamics of C partitioning and allocation (Hartmann et al., 2020; Hennion et al., 2019). Arbuscular mycorrhiza (AM) is an ubiquitous plant-microbial interaction established among a wide array of plant species and a monophyletic group of fungi that cluster in the subphylum Glomeromycotina (Spatafora et al., 2016).

Arbuscular mycorrhizal fungi (AMF) are heterotrophic organisms that require mycorrhizal associations with plants to complete their life cycle. These fungi develop within cortical cells, forming dichotomously branched structures called arbuscules, which are considered the main site of nutrient exchange between the AMF and the plant (Genre et al., 2020). The arbuscules are surrounded by a periarbuscular membrane that originates as an extension of the plasma membrane of the cortical cell; the apoplastic space between the periarbuscular membrane and the arbuscule is called the periarbuscular space (Gutjahr & Parniske, 2013; Ivanov et al., 2019).

When a mycorrhizal interaction is established, AMF obtain C from the plant in the form of carbohydrates and lipids derived from fatty acids (FAs) such as 2-monoacylglycerol (Jiang et al., 2017; Luginbuehl et al., 2017; Salmeron-Santiago et al., 2022; Wipf et al., 2019). In return, the fungus improves the efficiency at which the plant obtains minerals and water from the soil (Kakouridis et al., 2022). In addition, the fungus increases the plants stress (i.e., biotic and abiotic) tolerance (Begum et al., 2019; Jung et al., 2012).

Even though AMF are generally recognized as plant growth promoters, there is a broad range of phenotypic responses that mycorrhizal interactions trigger in plants. When plant growth improves as a result of AMF colonization, it is considered a positive phenotypic response. A response is considered neutral when the mycorrhiza do not cause significant changes in plant growth. Conversely, a negative phenotypic plant response is exhibited when plant growth is repressed (Lü et al., 2018; Smith et al., 2011). Recently, molecular analyses of a negative/neutral host response to AMF suggested that AMF could either be sensed by the host as a pathogen or that the AMF may be influencing other physiological responses, such as the hosts immunity (Cope et al., 2022).

AMF can enhance plants mineral uptake from the soil, such as nitrogen, phosphorus (P), potassium, and micronutrients. This enhancement is believed to be a key factor promoting plant growth (Chen et al., 2018; Liu et al., 2019; Ruytinx et al., 2020; Smith et al., 2011; Wang et al., 2020; Xu et al., 2007). Notably, AMF colonization is highly responsive to P availability; its uptake by the plant (i.e., via a mycorrhizal-dependent pathway) is an adaptive response to cope with Low P levels in soils (Chiu & Paszkowski, 2019; Ferrol et al., 2019).

Across different plant models, AMF colonization is limited or inhibited when plants grow under sufficient P levels. In contrast, Low P levels facilitate AMF colonization in the roots and trigger gene expression that underpins molecular mechanisms supporting plant P uptake through AM-dependent pathways (Balzergue et al., 2013; Ferrol et al., 2019; Nouri et al., 2014; Xu et al., 2007). However, AM symbiosis established under low P conditions has been shown to yield plants exhibiting negative phenotypes (for review, see Janos, 2007).

Therefore, plant growth enhancement by AMF is a multifactorial process. For example, a metadata analysis in mycorrhizal legumes revealed that enhancement of nutrient uptake via AM-dependent pathways does not fully explain the promotion of plant growth (Kaschuk et al., 2009). Instead, stimulation of sink activity that increases the harvest index (i.e., seed production relative to total shoot dry weight [DW]) has been proposed as a mechanism determining growth promotion of mycorrhizal plants (Kaschuk et al., 2009). Overall, this points to the possibility that C partitioning and allocation also play a role in promoting the growth of mycorrhizal plants (Saia, Tamayo, et al., 2020).

The AMF carbon requirements are met via carbohydrates transported from the source tissues to the sink (i.e., colonized roots) (Gutjahr et al., 2011). Regarding FA, Suc and its catabolic products are the main sources of C that support FA biosynthesis in plant tissues (Lim et al., 2017; Ma et al., 2017). Previous studies have revealed the existence of molecular mechanisms that modulate the mycorrhizal development by controlling the amount of Suc and monosaccharides in the periarbuscular space, mediated by SUT and SWEET transporters (An et al., 2019; Bitterlich et al., 2014; Tamayo et al., 2022).

However, C partitioning and allocation in both AMF and the host plant during mycorrhizal symbiosis, as well as the molecular mechanisms contributing to the sink strength, involving sugar metabolism, are still poorly understood. Furthermore, there is limited information on the relationship between C metabolism and mycorrhizal performance under contrasting growth responses of the host plant.

We hypothesize that the amount and source of C invested in AM by the plant will depend on P availability and will determine the plants phenotypic outcomes and mycorrhizal performance. To gain insights into C dynamics in response to AM symbiosis, we manipulated the P available to *S. lycorpersicum* plants inoculated with *Rhizophagus irregularis* to promote positive (Low P, 0.065 mM), neutral (High P, 1.3 mM), and negative (Null P, 0 mM) phenotypic outcomes. We then quantified the performance of mycorrhizal symbiosis by staining AMF structures and the lipid biomarker 16:1ω5 within the roots. We then determined the number of spores in the substrate and the accumulation of mycorrhizal gene markers. Finally, we also analyzed the pattern of soluble sugar and starch content in source leaves and roots in response to AMF inoculation and the expression of genes that control sink activity in mycorrhizal roots across the three contrasting phenotypic outcomes.

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2 | MATERIALS AND METHODS

2.1 | Plant and AMF models, experimental design, and culture conditions

The fungus *R. irregularis* (DAOM 197198) was provided by the CIDIIR-IPN, Unidad Sinaloa, as spores obtained from monoxenic cultures (Maldonado-Mendoza & Harrison, 2018), and was propagated on tomato plants (*Solanum lycorpersicum* cv. Río Grande) grown in sterile sand during eleven weeks under greenhouse conditions. The inoculum consisted of spores, colonized root fragments and mycelia in sand. The macronutrient content of the mycorrhizal inoculum in milligrams per kilogram (mg kg^{-1}) corresponded to 35.8, P (Bray extractable P): 5.72, N-NO_3: 21.3, K: 345, Ca: 12.3, Mg: 105, Na; and 9.26, S.

To perform the experiments, tomato seeds (*S. lycorpersicum* cv. Río Grande) were superficially disinfected in a 5% commercial solution of sodium hypochlorite (i.e., for 10 min) and rinsed five times with sterile distilled water. The seeds were then placed in tyndallized peat and watered with distilled water until they germinated. After 15 days, seedlings with expanded cotyledons were transplanted into nursery bags and kept under greenhouse conditions. Prior to transplantation, arbuscular mycorrhizal inoculation was performed by adding 150 g of mycorrhizal inoculum in nursery bags filled with 1 L of tyndallized silica sand. Non-colonized controls were supplemented with 150 g of the same mycorrhizal inoculant that had been tyndallized to eliminate mycorrhizal propagules. After transplantation, non-inoculated controls were supplemented with 50 ml of a mycorrhizal inoculum suspension (i.e., 10 g of inoculum suspended in 90 ml of distilled water) filtered through a 37 μm sieve and cotton fibber, to discard mycorrhizal propagules. Thus, non-inoculated control plants were grown on the same substrate as inoculated plants minus the AMF (same physicochemical features and the biological components of the inoculant).

The colonized and non-colonized plants were fertilized twice a week with 25 ml of Long-Ashton nutrient solution with different concentrations of monobasic sodium phosphate. These treatments were named and will subsequently be referred to as Null P, Low P, and High P, corresponding to 0, 0.065, and 1.3 mM NaH_2PO_4, respectively. The latter concentration of NaH_2PO_4 is the one originally described as the source of inorganic phosphate (Pi) for the Long-Ashton nutrient solution (Smith et al., 1983). The plants were watered with 25–50 ml of distilled water when necessary.

Tomato plants were kept under greenhouse conditions for 8 weeks after transplanting, arranged in a completely randomized design consisting of six treatments, each with five biological replicates. In addition, each biological replicate consisted of a nursery bag containing one tomato plant from each treatment group.

2.2 | Nondestructive morphometric and physiological determinations and plant harvesting

Morphometric and physiological variables of 10-week-old inoculated and non-inoculated tomato plants were quantified. The height (cm), stem diameter (mm), and root length (cm) were measured. The biovolume index was calculated using a formula proposed by Hatchel et al. (1985). The root volume (cm^3) was determined via water displacement in a graduated cylinder. Total shoot and root fresh weights (FW) (g) were also recorded. The relative chlorophyll content was estimated as SPAD units using a Minolta SPAD-502 (Konica Minolta Optics, Inc.). Leaf area and SPAD units were calculated using the two youngest, fully expanded leaves closest to the shoot apex. These two leaves will subsequently be referred to as source leaf 1 (SL1) and SL 2 (SL2), corresponding to the youngest and the second youngest expanded leaves, respectively (Figure S1A). The leaf areas of SL1 and SL2 were estimated using digital photographs inputted into the image J software (Schneider et al., 2012).

SL1 and SL2 leaflets and root aliquots from all biological replicates per treatment were cut and snap-frozen in liquid nitrogen during the 9:00 a.m. to 11:00 a.m. light period and subsequently stored at −80°C. The rest of the plant tissues were weighed fresh and then dried at 70°C (48 h) to determine the DW of shoots and roots.

2.3 | Quantification of mycorrhizal colonization of roots and spore content of the substrate

To visualize AM colonization, dried tomato roots were stained using a modified Phillips and Hayman (1970) protocol. The roots were hydrolyzed by being autoclaved at 121°C in 10% KOH (i.e., for 10 min). After decanting the KOH solution, the roots were rinsed three times with distilled water and cleared with 1% HCl (1 min); then 0.05% trypan blue in acetoglycerol (27 ml acetic acid; 33 ml glycerol; 40 ml H_2O) was added and the roots were autoclaved again at 121°C (10 min). Thirty-six 1-cm-long root fragments from three biological replicates of each treatment were then mounted on slides and observed under an optical microscope to determine the extent of mycorrhizal colonization and quantify arbuscules and vesicles according to Trouvelot et al. (1896).

AMF spores were extracted from the substrate in which the plants grew during the experiment. Spores were extracted via wet sieving and decantation (Gerdemann & Nicolson, 1963) followed by centrifugation (595 g, 1 min) with 46% Suc. The number of spores per 50 g of substrate was expressed as the mean of three independent replicates.

2.4 | Quantification of the AM biomarker lipid 16:1ω5

Quantification of the AM lipid 16:1ω5, which is a quantitative biomarker to estimate AMF biomass, was performed in at least three replicates of each treatment from inoculated and non-inoculated roots using gas chromatography (GC) with the Microbial Identification System (MIDI, Inc.). The results were expressed as nanomoles of the AM biomarker lipid 16:1ω5 per gram of FW (nM g FW). Whole-cell FA (WCFA) were extracted from root samples using a four-step FA extraction method described by Sasser (Sasser, 1990). The method

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entails the following steps: (1) saponification, (2) methylation, (3) extraction, and (4) base wash. To quantify the extracted FA methyl esters, an internal standard, non-adeconoate FA methyl ester, was added to each sample. Then, 25 mg of freeze-dried root samples were saponified in 2 ml of NaOH in 50% methanol in a glass vial placed in a 100°C water bath for 5 min. The glass vial was vigorously vortexed for 10 s and returned to the water bath for an additional 25 min. After cooling, the samples were methylated by adding 4 ml of a methylation solution (325 ml of 6 N HCl + 275 ml of 99.9% methanol) and placed in a water bath at 80°C for 10 min. FA methyl esters were extracted by adding 2.5 ml of a 50% hexane:50% methyl *tert*-butyl ether solution and mixing the vials for 5 min in a rotary shaker, followed by removal of the lower aqueous phase. The organic extract was then washed with 6 ml of a 0.3 M solution of NaOH in H_2O to remove non-methylated FA and other residues. The organic phase was then transferred to a different glass vial and capped.

FA methyl ester analyses were performed using the Sherlock software package (version 6.0; MIDI Inc.) and an HP6890 GC fitted with a 25-m fused silica capillary column (HP part no. 19091B-102) and using hydrogen as the carrier gas. The injector temperature was 250°C, whereas the detector temperature was 300°C. The temperature program was as follows: Initial temperature of 170°C increasing to 270°C at 5°C/min. A total of 2 µl of each sample was then injected. The MIDI software automatically controlled all GC operations, including calibration, subsequent sample sequencing, peak integration, and naming. The calibration standards contained a mixture of straight-chain saturated and hydroxy FA methyl esters with a length of 10–20°C (MIDI part mo. 1200A).

2.5 | Soluble sugar and starch content

Frozen plant tissues were first pulverized in a mortar with liquid nitrogen. Ground aliquots of each sample (25–50 mg) were mixed with 500 µl of 80% ethanol (v/v) and incubated at 80°C (30 min). The mixture was then centrifuged at 14,875 × *g* (15 min) and the supernatant was collected in a 2 ml tube. The aqueous-ethanolic extraction steps were repeated four times, and the supernatants were collected in a single tube per sample. Subsequently, 80% ethanol was evaporated by incubation at 60°C (12–15 h), and the contents of the tube were resuspended in 1 ml distilled water, centrifuged as described above, and transferred to a new tube; finally, these steps yielded the soluble sugar extract (SSE). The mass debris of each 25–50 mg sample was further extracted twice with 80% ethanol, and then the debris pellet was dried and stored for starch quantification.

The total soluble sugars were quantified using the phenol-sulfuric acid method (DuBois et al., 1956). The reaction mixture consisted of 50 µl SSE, 80 µl of 5% phenol, and 500 µl of 98% sulfuric acid. Distilled water was then added to a final volume of 780 µl. The reactions were incubated at room temperature (15 min) and their absorbance was measured at 490 nm. Total soluble sugars were estimated from a calibration curve using glucose (Glc) as a standard and were reported as µg Glc mg^{-1} FW.

Total reducing sugars were quantified using the Somogyi–Nelson reagents (Nelson, 1944; Somogyi, 1952). The reaction mixture consisted of 50 µl of SSE mixed with 400 µl of Somogy's copper reagent and distilled water to a final volume of 600 µl. Reactions were incubated at 100°C (45 min) and then cooled at room temperature in the dark (30 min). Nelson's reagent (400 µl) was then added to a final volume of 1 ml, and the reactions were incubated in the dark at room temperature for 30 min. Absorbance was measured at 550 nm, and a calibration curve was constructed using Glc as a standard. Reducing sugars were reported as µg Glc mg^{-1} FW.

The total ketose sugars were quantified using the resorcinol reagent method (Roe, 1934). The reaction mixture consisted of 50 µl of SSE, 100 µl resorcinol reagent (0.1% resorcinol; diluted in 96% ethanol), and 700 µl of concentrated HCl. Distilled water was then added to a final volume of 1 ml. Reactions were incubated at 80°C (15 min) and cooled at room temperature (15 min). Absorbance was measured at 520 nm, and the total ketose sugars content was estimated from a calibration curve using fructose as the standard and expressed as µg Fru mg^{-1} FW.

Nonreducing ketoses were quantified using the resorcinol reagent. A 50 µl aliquot of SSE was first incubated at 100°C with 0.2 N NaOH to a final volume of 400 µl to hydrolyze free hexoses. Reactions were mixed with 100 µl resorcinol reagent and 500 µl of HCl, then incubated at 80°C (15 min). Absorbance was measured at 520 nm, and the total nonreducing ketose sugar content was estimated from a calibration curve using Suc as the standard and reported as µg Suc mg^{-1} FW.

Reducing ketoses were estimated by subtracting the calculated molar fructose equivalents in the fraction of nonreducing ketoses from the total ketoses; the result is expressed in mass units as µg Fru mg^{-1} FW.

Starch was quantified according to a procedure reported by Smith and Zeeman (2006) with the following modifications: dry, ethanol-extracted debris pellets were resuspended in 200 µl of distilled water, heated to 100°C (45 min), then cooled to room temperature (15 min). Subsequently, 300 µl of a reaction mixture consisting of 1.7 µl of α-amylase (Sigma A3403) and 1.1 µl of α-amyloglucosidase (Sigma A1602) in 0.2 M sodium acetate buffer (pH 5.4) were added. Reactions were incubated at 37°C (24 h). Reducing sugars were quantified from a 50 µl aliquot of a 1:10 dilution of enzymatically hydrolyzed starch with Somogyi–Nelson's reagents, as described above. Starch content was estimated from a calibration curve using Glc as a standard and was expressed as µg Glc mg^{-1} FW. The total soluble sugar-to-starch ratios were calculated as the amount of total soluble sugars per starch content based on individual leaf and root samples.

2.6 | Gene expression analysis via qRT-PCR

For gene expression analysis, total RNA was extracted from ground aliquots of root tissues using the PureLink RNA Mini Kit (Invitrogen).

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cDNA synthesis was performed using 400 ng of total RNA from each sample using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems), and all cDNA synthesis reactions were performed in a final volume of 20 µl. The RNA extraction and cDNA synthesis kits were used according to the manufacturer's protocol.

For qPCR, the reaction mixtures consisted of 0.4 µM of each oligonucleotide, 5 µl of TB Green Premix Ex Taq (Tli RNaseH Plus), 1 µl of a 1:20 dilution of each cDNA sample, and nuclease-free H_2O to a final volume of 10 µl. Four independent biological replicates per treatment were analyzed. The amplification program established in a StepOne Real-Time PCR System (Applied Biosystems) was 95°C, 30 s, followed by 40 cycles of 95°C, 5 s and 58°C, 30 s. Relative quantification was performed using the comparative $2^{-\Delta(\Delta Ct)}$ method (Livak & Schmittgen, 2001). Gene expression was normalized to that of the housekeeping gene, elongation factor-1α (*SlEF-1α*). The oligonucleotide sequences of the analyzed genes are listed in Table S1.

2.7 | Statistical analyses

Statistical analyses were performed using the SAS OnDemand for Academics software (SAS Institute Inc.). At least three independent replicates per treatment were analyzed. The normality of the data was evaluated using the Shapiro–Wilk test and the homogeneity of variance was confirmed using the Levene test. Data that met normality and homoscedasticity were compared using the Student's *t* test or analysis of variance (ANOVA) using ANOVA or GLM procedures when data were balanced or unbalanced, respectively, followed by comparisons of Fischer's means or Tukey's test ($p < 0.05$), respectively. The Pearson correlation coefficient was calculated using the PROC CORR (SAS) procedure.

To compare the influence of NaH_2PO_4 concentration and mycorrhizal colonization across the six treatments, we performed a two-factor ANOVA comparison, considering NaH_2PO_4 conditions (i.e., Null, Low, and High P) and *R. irregularis* colonization (presence or absence). These analyses were followed by Tukey's comparison of the means ($p < 0.05$).

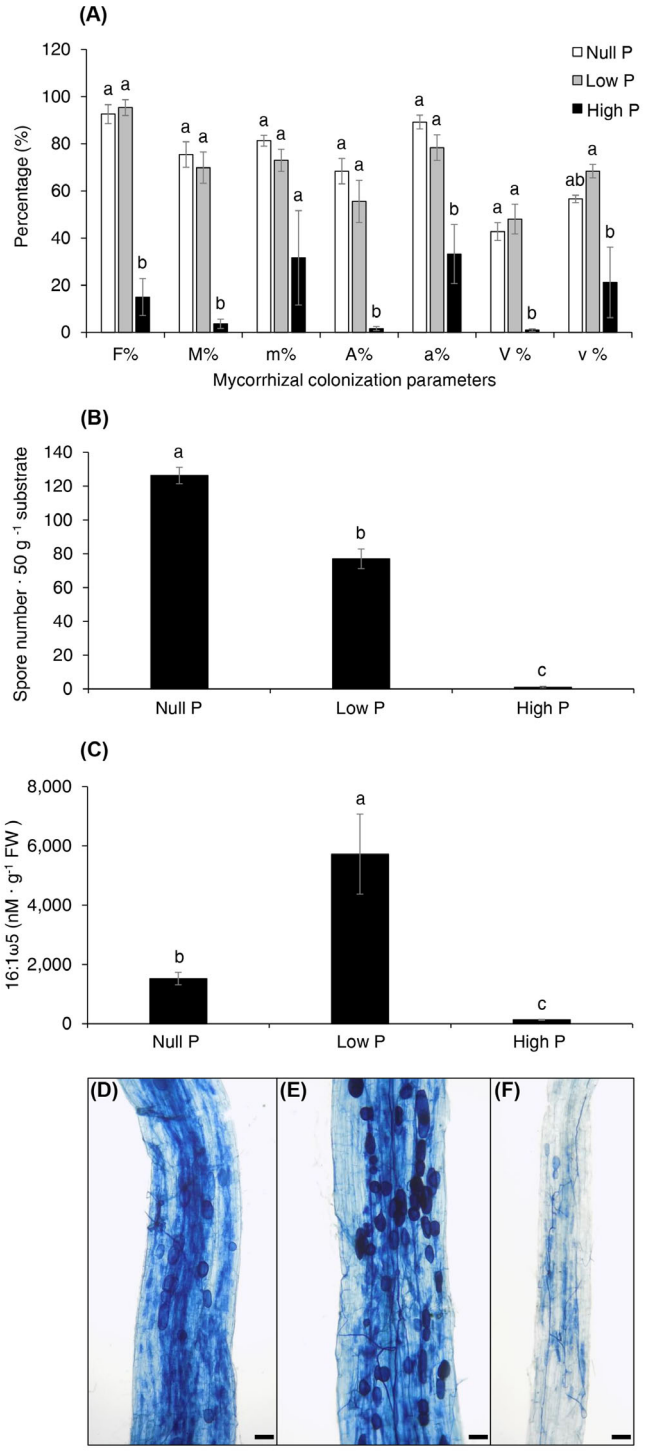

FIGURE 1 Mycorrhizal colonization of tomato plants under three P fertilization conditions. (A) Intrarradical arbuscular mycorrhiza (AM) quantification according to Trouvelot et al. (1896) parameters. (B) *Rhizophagus irregularis* spore density in the substrate. (C) Root content of AM biomarker lipid 16:1ω5. (D–F) Mycorrhizal colonization as visualized under Null P (D), Low P (E), and High P (F). *F%*, frequency of mycorrhizal colonization; *M%*, the intensity of mycorrhizal colonization in the root system; m%, the intensity of mycorrhizal colonization in root fragments; *A%*, arbuscule abundance in the root system; a%, arbuscule abundance in colonized root fragments; *V%*, vesicle abundance in the root system; *v%*, vesicle abundance in colonized root fragments. Data represent the average of at least three replicates; significance was determined by analysis of variance (ANOVA) (A and B) or by the GLM procedure (C); error bars are the standard error of the mean; different letters indicate statistically significant differences according to Tukey's test ($p < 0.05$). Scale bar represents 50 µm (D–F); Null P, Low P, and High P conditions represent Long Ashton's nutrient fertilization under three NaH_2PO_4 concentrations (0, 0.065, and 1.3 mM, respectively).

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3 | RESULTS

3.1 | Intraradical AMF biomass and spore yield accumulate differentially under contrasting P levels

To determine if P levels translate to contrasting levels of mycorrhizal colonization in plants, we set up a preliminary experiment where tomato plants were inoculated with *R. irregularis* and grown under six different concentrations of NaH_2PO_4. The concentrations ranged from 0 to 1.3 mM. Our results showed that 0, 0.065, and 1.3 mM NaH_2PO_4, designated here as Null P, Low P, and High P, respectively, resulted in different patterns of mycorrhizal colonization. A modification of the osmotic potential of the Long-Ashton nutrient solution by reducing the P source did not significantly change the colonization levels in inoculated plants (Figure S2).

To analyze the performance of AM colonization under the three P fertilization conditions, we compared the quantification of intraradical mycorrhizal structures according to Trouvelot et al. (1896), the accumulation of AMF biomass by quantifying 16:1ω5 in roots, and the content of spores in the substrate of inoculated tomato plants.

The inoculated plants subjected to Null and Low P showed a high percentage of colonization across all measured mycorrhizal parameters, presenting more than 60% intraradical mycorrhizal colonization intensity (*M*%) and more than 50% arbuscule abundance (*A*%), without significant differences between plants under the two P conditions. On the other hand, mycorrhizal colonization parameters were significantly reduced in plants grown under High P. The mycorrhizal colonization intensity within the colonized root fragments (m%) was the only parameter that showed similar results among the three P conditions (Figure 1A). Spore quantification was negatively correlated with P concentration ($r^2 = -0.93031$; $p < 0.05$). The substrate spore count of Null P plants was significantly ($p < 0.05$) higher than that of Low P and High P plants (Figure 1B).

The accumulation of the neutral lipid FA 16:1ω5 showed a high and negative Pearson's correlation coefficient with the reduction of P concentration ($r^2 = -0.77816$; $p < 0.05$), suggesting that the reduction of P is positively correlated with an increase in AMF biomass accumulation. However, the accumulation of 16:1ω5 in inoculated roots under Low P was 3.5 and 40 times higher than that under Null P and High P, respectively (Figure 1C). A higher proportion of vesicles was quantified in root fragments (*v*%), and visual observations were consistent with this measurement (Figure 1D–F), although it was not significantly different from the v% quantified under Null P conditions.

3.2 | Phosphate availability modulates host phenotypic response to AMs

The phenotypic response of tomatoes to *R. irregularis* under the three P conditions was characterized. The phenotypic outcomes of the 10-week-old tomato plants were significantly different under the three P fertilization conditions (Figure S1B). High P plants had the most positively affected morphometric and physiological outcomes, while all measures of development were significantly reduced ($p < 0.05$) in plants under Low and Null P (Figure 2). Additionally, by decreasing the level of P fertilization in Null P and Low P plants, the values of shoot DW, root DW, and total DW were significantly reduced ($p < 0.05$), despite not being significantly different between inoculated and non-inoculated plants at each level of P fertilization (Table 1).

Mycorrhizal colonization under Null P significantly ($p < 0.05$) reduced plant height of colonized plants by 0.73 times, the biovolume index decreased by 0.72 times, and the leaf area of SL1 was reduced by 0.7 times compared to non-colonized plants (Figure 2A–C). The relative amount of chlorophyll in SL1 and SL2, measured as SPAD units, showed a significant ($p < 0.05$) increase of at least six units in both source leaves at Null P (Figure 2E,F).

Low P conditions reversed the growth suppression exerted by mycorrhizal treatment observed in Null P plants. Inoculated plants under Low P showed a 1.3-fold increase in the SL1 area compared to non-inoculated plants under the same P level (Figure 2C). However, no significant differences ($p < 0.05$) were found in the SPAD units of the source leaves (SL1 and SL2) between inoculated and non-inoculated plants at Low P.

Inoculated plants under High P showed statistically similar morphometric and physiological variables to non-inoculated plants, except for SL1 SPAD units, which increased by three units in the mycorrhizal treatment ($p < 0.05$) (Figure 2E).

Root developmental parameters were altered by P availability; however, mycorrhizal colonization did not significantly modify root development under any P condition. Root volume was significantly ($p < 0.05$) decreased by P reduction in the nutrient solution in Null P and Low P plants. Root length was statistically similar in all treatments, but the ratio of root length to shoot height was greater in Null P and Low P plants than in High P plants, indicating changes in root architecture under P deficiency (Figure S3).

3.3 | Partitioning and allocation of soluble sugars and starch in source and sink tissues are modified by P availability and mycorrhizal colonization

To determine whether carbohydrate partitioning and allocation in plants are altered under the three levels of P availability and mycorrhizal colonization intensities, fractions of soluble sugar and starch content in the source leaves and roots were quantified.

Starch content in the source leaves was not significantly altered by mycorrhizal colonization at any P level (Figure 3A). However, the source leaves of all inoculated plants showed significantly higher ($p < 0.05$) soluble sugar content than non-inoculated plants, regardless of the P condition (Figure 3B).

Mycorrhizal colonization under Null P significantly ($p < 0.05$) promoted an increase in total reducing sugars by 1.3 times more than in non-inoculated plants, as well as the content of both total ketoses and total reducing ketoses, which increased 1.2 times in inoculated plants (Figure 3C,D,F); however, no significant differences were

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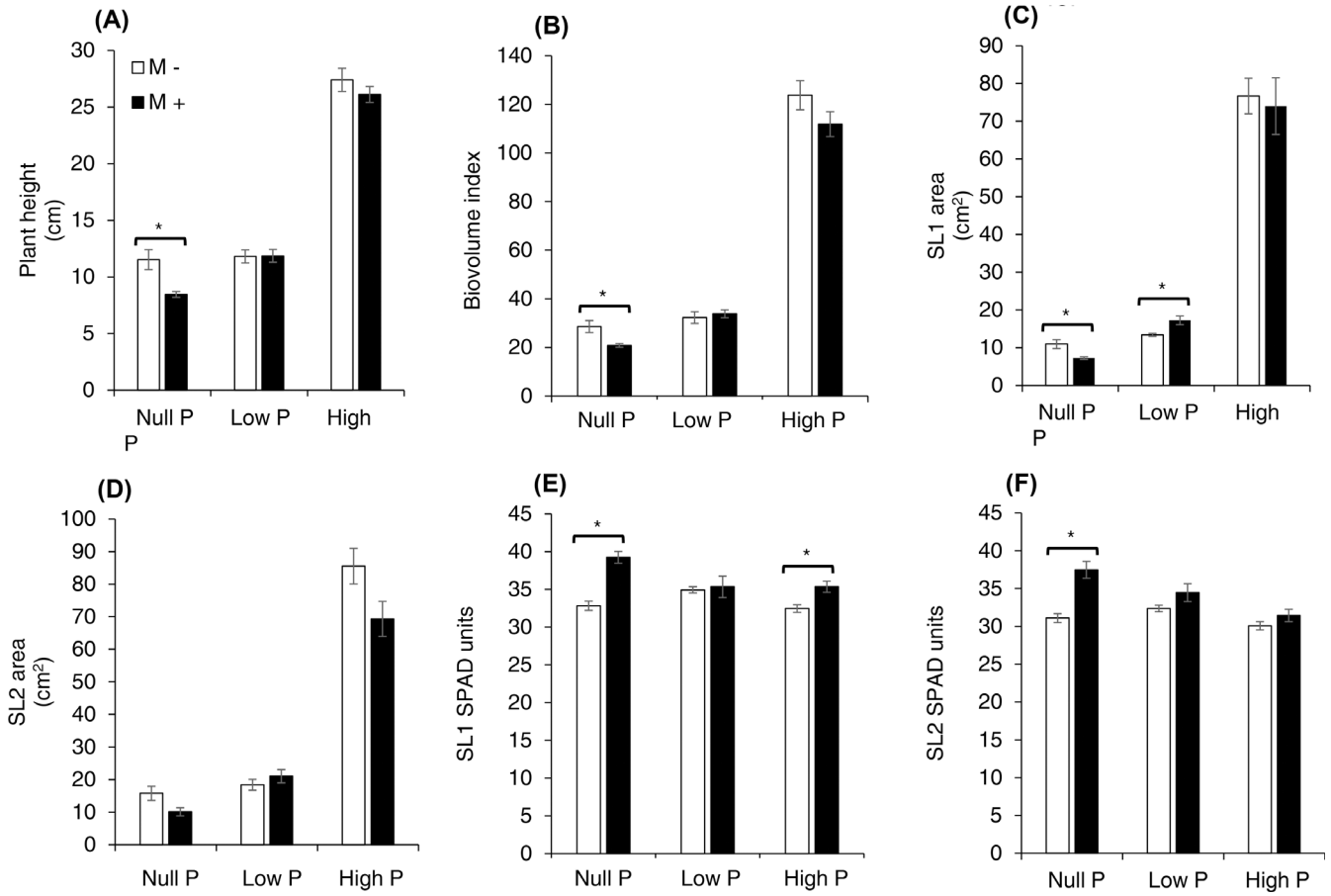

FIGURE 2 Tomato shoot and leaf responses to mycorrhizal colonization under different P fertilization conditions. (A) Data represent the average of five replicates for plant height; (B) biovolume index; (C, D) leaf area of SL1 and SL2, respectively; and (E, F) the relative content of chlorophyll expressed as SPAD units corresponding to SL1 and SL2. Error bars are the standard error of the mean; the asterisk indicates significant differences between M− and M+ plants for each P fertilization level (*t* test, $p < 0.05$). M−, non-mycorrhizal plants; M+, mycorrhizal plants; Null P, Low P, and High P conditions represent Long Ashton's nutrient fertilization under three NaH_2PO_4 concentrations (0, 0.065, and 1.3 mM, respectively).

TABLE 1 The dry weight of non-mycorrhizal (M−) and mycorrhizal (M+) plants. Data represent the average of five replicates; significance was determined by ANOVA; (±) are the standard error of the mean; different letters indicate significant difference according to Tukey's test ($p < 0.05$)

NaH_2PO_4 concentration	*Rhizophagus irregularis* inoculation	Shoot DW	Root DW	Total DW
Null P	M−	0.173 (±0.025) b	0.119 (±0.017) b	0.292 (±0.041) b
	M+	0.126 (±0.014) b	0.098 (±0.018) b	0.224 (±0.030) b
Low P	M−	0.205 (±0.020) b	0.136 (±0.047) b	0.342 (±0.066) b
	M+	0.213 (±0.019) b	0.219 (±0.041) b	0.432 (±0.052) b
High P	M−	1.322 (±0.057) a	3.543 (±0.518) a	4.865 (±0.575) a
	M+	1.279 (±0.068) a	2.384 (±0.485) a	3.66 (±0.522) a

Abbreviations: ANOVA, analysis of variance; DW, dry weight.

observed in nonreducing ketoses (Figure 3E). All soluble sugar fractions quantified in the source leaves were statistically similar across inoculated and non-inoculated plants under Low and High P conditions (Figure 3C–F).

As observed in the source leaves, no differences were found in the root starch content of inoculated and non-inoculated plants under any P levels (Figure 4A). However, while total soluble sugars and total reducing sugars were statistically similar in mycorrhizal roots in the

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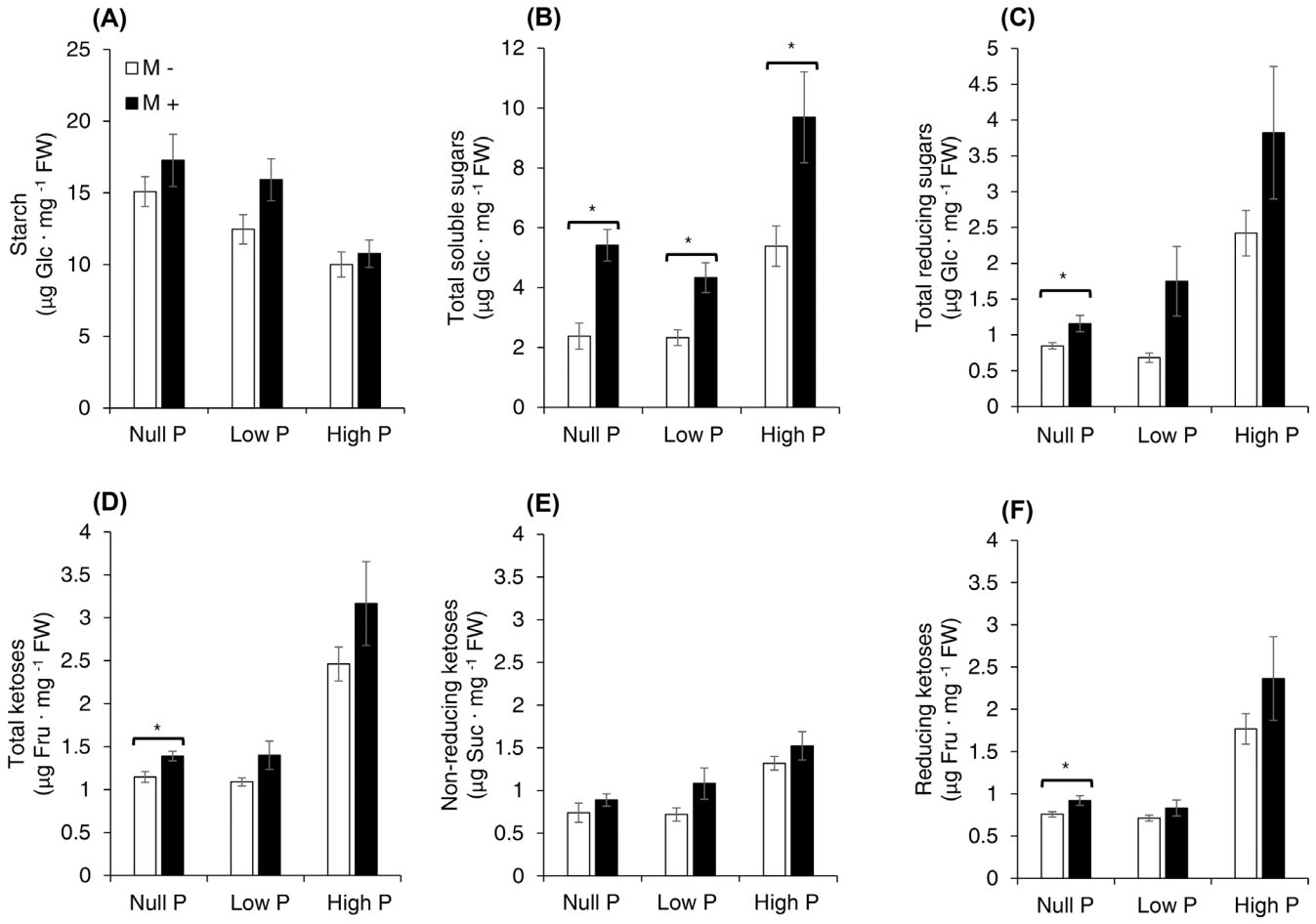

FIGURE 3 Effect of P availability and mycorrhizal conditions on starch and soluble sugar content in source leaves. (A) Data represent the average of five replicates corresponding to starch, (B) total soluble sugars, (C) total reducing sugars, (D) total ketoses, (E) nonreducing ketoses, and (D) reducing ketoses. Error bars are the standard error of the mean; the asterisks indicate significant differences between M− and M+ plants for each P fertilization level (*t* test, $p < 0.05$). M−, non-mycorrhizal plants; M+, mycorrhizal plants; Null P, Low P, and High P conditions represent Long Ashton's nutrient fertilization under three NaH_2PO_4 concentrations (0, 0.065, and 1.3 mM, respectively).

Null P and High P treatments compared to non-inoculated plants under the same P conditions, mycorrhizal colonization under Low P conditions caused a decrease in the content of soluble sugars in the roots. Under Low P, total soluble sugars, total reducing sugars, and reducing ketoses in roots were 1.69, 1.84, and 1.28 times higher in non-inoculated than in inoculated plants, respectively (Figure 4B,C,F). Under Null P, the roots of the non-inoculated plants showed a 1.25-fold increase in total ketoses compared to the inoculated plants (Figure 4D). However, the reduction in total ketoses in the roots of inoculated plants did not significantly modify the accumulation of total soluble sugars compared with non-inoculated plants (Figure 4B).

To further explore the effect of P availability and mycorrhizal colonization on carbohydrate partitioning and allocation in plants, the ratio of total soluble sugars to starch in the source leaves and roots was quantified and it was higher in inoculated plants than in non-inoculated plants under High P conditions (Figure S4A). Mycorrhizal colonization increased the ratio of total soluble sugars to starch in source leaves (Figure S4B), whereas the total soluble sugar-to-starch ratio in source leaves and roots under Null P and Low P was lower than that under High P (Figure S4C). Mycorrhizal colonization did not significantly change the ratio of total soluble sugar to starch in the roots of plants grown under any P level (Figure S4D). These results were consistent when inoculated and non-inoculated plants were compared, regardless of the amount of P applied during fertilization (Figure S4E). As in the source leaves, the ratio of total soluble sugars to starch in the roots of plants under Null P and Low P was reduced (Figure S4F).

We also observed that plants under P starvation allocated a greater amount of C to starch biosynthesis, reducing the total soluble sugar to starch ratio in both, source and sink tissues (Figures 3A and 4A). Regardless of the effect of P starvation on C accumulation as starch in source leaves, AM promoted C allocation to the biosynthesis of soluble sugars, which can subsequently be partitioned to the mycorrhizal roots. Therefore, carbon allocation to soluble sugars in inoculated plants seems not to be limited by starch accumulation in source leaves caused by P deficiency under Null and Low P conditions.

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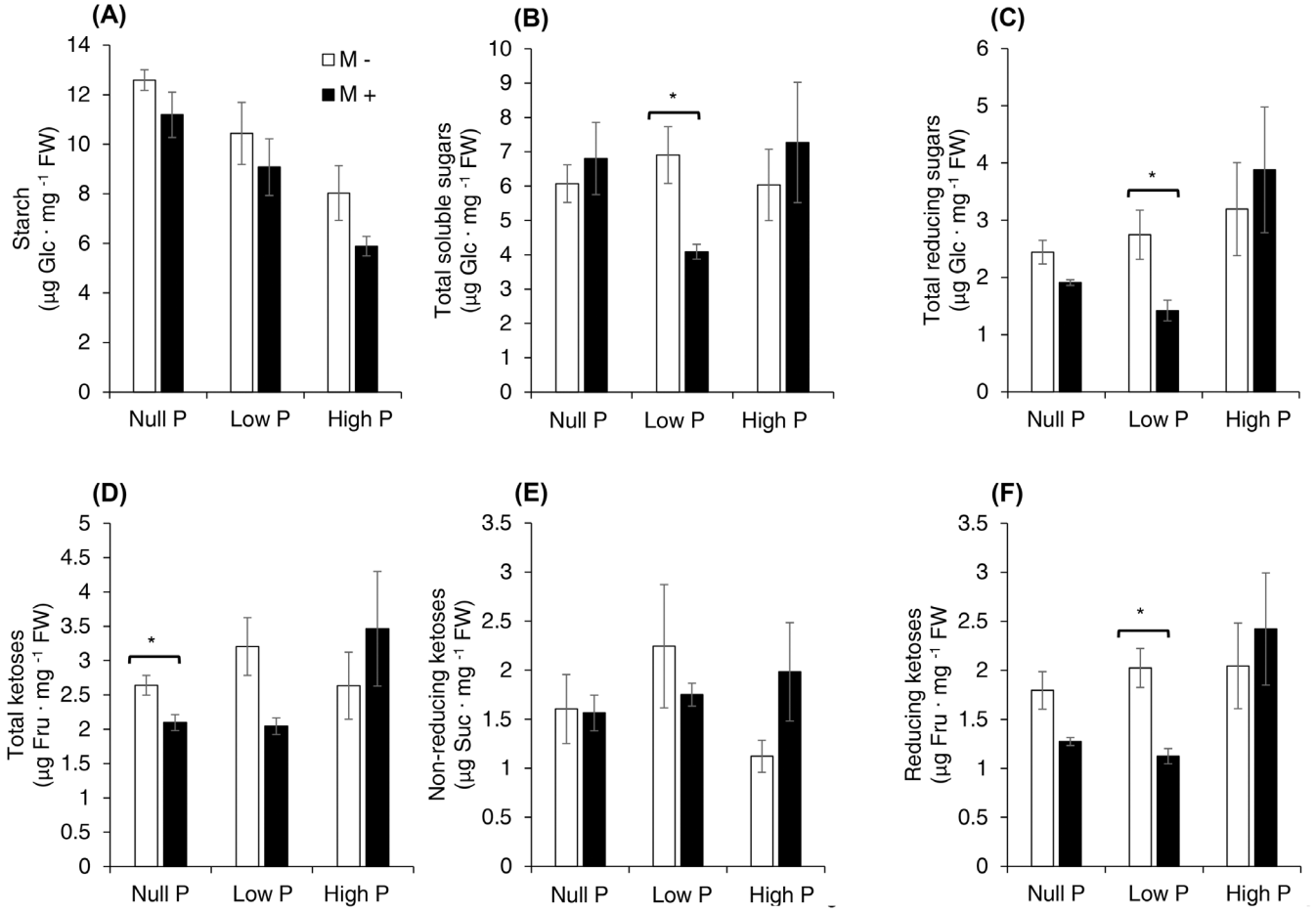

FIGURE 4 Effect of P availability and mycorrhizal conditions on starch and soluble sugar content in tomato roots. Data represent the average of three replicates corresponding to (A) starch, (B) total soluble sugars, (C) total reducing sugars, (D) total ketoses, (E) nonreducing ketoses, and (F) reducing ketoses. Error bars are the standard error of the mean; the asterisks indicate significant differences between M− and M+ plants for each P fertilization level (*t* test, $p < 0.05$). M−, non-mycorrhizal plants; M+, mycorrhizal plants; Null P, Low P, and High P conditions represent Long Ashton's nutrient fertilization under three NaH_2PO_4 concentrations (0, 0.065, and 1.3 mM, respectively).

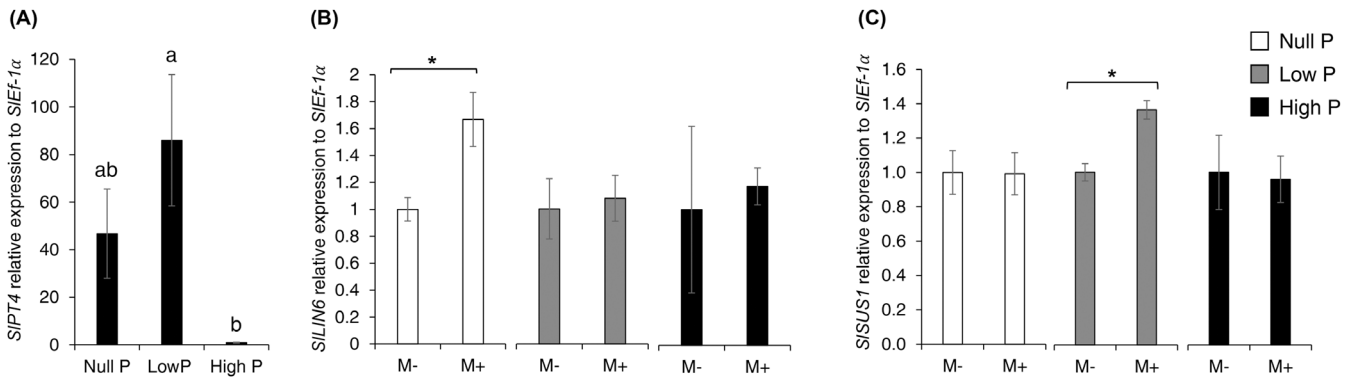

FIGURE 5 Gene expression analysis in roots of the arbuscule-specific P transporter and genes encoding enzymes that determine C partitioning. Levels of expression of the (A) phosphate transporter *SlPT4*, (B) cell-wall bound invertase *SlLIN6*, and (C) cytoplasmic sucrose synthase *SlSUS1*. The expression levels were normalized to the expression of the housekeeping gene *SlEf-1α* and to a sample corresponding to the highest P concentration (A) or to non-mycorrhizal plants from each P condition tested (B and C). Data represent the average of at least three replicates; error bars are the relative error of the mean. Data were compared by the GLM procedure (A) or by the Student's *t* test (B and C). Different letters indicate statistical differences according to Tukey's test ($p < 0.05$) and the asterisk indicates significant differences between M− and M+ plants for each P fertilization level (*t* test, $p < 0.05$). M−, non-mycorrhizal plants; M+, mycorrhizal plants; Null P, Low P, and High P conditions represent Long Ashton's nutrient fertilization under three NaH_2PO_4 concentrations (0, 0.065, and 1.3 mM, respectively).

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3.4 | Upregulation of sink strength gene markers revealed that mycorrhizal symbiosis mediates C allocation to the host

To evaluate the extent of symbiosis across the various treatments, we analyzed the expression of *SlPT4* in the roots, an arbuscule-specific P transporter that mediates P uptake via the mycorrhizal pathway. The highest expression of *SlPT4* was observed in plants grown under Low P, followed by plants grown under Null P (Figure 5A). *SlPT4* expression was markedly reduced in plants exposed to the highest concentration of P (Figure 5A) and was not detected in root samples from non-inoculated plants.

To explore the molecular mechanisms mediating sink strength and C allocation in mycorrhizal roots, we analyzed the expression of genes encoding enzymes involved in Suc catabolism, a cell wall-bound invertase (*SlLIN6*), and a cytoplasmic Suc synthase (*SlSUS1*). *SlLIN6* expression was significantly higher ($p < 0.05$) in inoculated plants grown under Null P than in non-inoculated plants grown under the same P conditions (Figure 5B). There were no significant differences in *SlLIN6* expression between inoculated and non-inoculated plants in the Low P and Null P groups. In contrast, the expression of *SlSUS1* was significantly higher ($p < 0.05$) in the roots of inoculated plants at Low P, while no detectable changes in the expression of this gene were observed between inoculated and non-inoculated plants grown under Null P and High P, respectively (Figure 5C).

4 | DISCUSSION

4.1 | Specific mycorrhizal phenotype of *S. lycorpersicum* as a function of P availability

Different degrees of colonization and plant growth responses, from positive to negative, have been previously described in mycorrhizal interactions. This spectrum of responses can be influenced by various factors, including the type of AM fungi and the species or variety of the host plant (Janos, 2007; Thirkell et al., 2022; Tran et al., 2019). The plant's phenotypic outcomes depend on the balance of benefits and costs of the plant-microbial interaction, which can be shaped by extrinsic conditions, including the availability of P (Smith et al., 2011). Here, we show that the tomato plant, *S. lycorpersicum*, responds to P fertilization levels for most of the measured morphological and physiological variables. Specifically, the impact of mycorrhizal colonization on plant phenotypes was dependent on the intensity of P fertilization, revealing a broad plant phenotypic response to AM symbiosis according to nutrient availability.

Plants not fertilized with P (Null P) only obtained P via the mycorrhizal inoculant. Under such conditions (i.e., limited P), the resultant phenotypic and physiological outcomes of non-inoculated plants were poor. However, Null P plants inoculated with *R. irregularis* showed abundant intraradical AM colonization but also exhibited negative growth outcomes (Figure 6). These results point to the possibility that mycorrhizal colonization could reduce plant growth under Null P due to P competition between AMF and the host plant. This reduction could be due to an increase in the sink strength of AMF colonized roots, while photosynthetic organs in young mycorrhizal plants are still poorly developed, further limiting C partitioning to the host plant (Janos, 2007). However, it has been previously reported that *S. lycorpersicum* seedlings fertilized with 32 μM of P and inoculated with *Funneliformis mosseae* have a better growth and a lower stress response than non-inoculated seedlings (Cesaro et al., 2020). It was previously proposed that negative plant growth responses to mycorrhizae in early development stages may be transient until plants develop photosynthetic tissues to ensure a steady supply of C (Janos, 2007). However, we observed a growth suppression of inoculated plants under P starvation even in plants with true leaves.

4.2 | Mycorrhizal colonization in contrasting plant growth phenotypes

The "aggressiveness" of AMF colonization has been proposed as a factor underlying the growth suppression of mycorrhizal plants. For example, there have been scenarios where AMF species that heavily colonize roots cause a reduction in the growth of wheat (*Triticum aestivum* cv. Kulin) under both Low and High P levels (Graham & Abbott, 2000). *R. irregularis* is characterized by a high root colonization capacity in short periods compared to other AMF species (Hart & Reader, 2002; Werner & Kiers, 2015). Here, we found that the proportion of intraradical AMF structures was high and similar in plants under Null P or Low P when quantified in root fragments. Similarly, high levels of colonization have been achieved in *Petunia hybrida* fertilized with KH_2PO_4 concentrations ranging from 0 to 0.1 mM (Nouri et al., 2014); this pattern was also observed for *Medicago truncatula* plants fertilized with 0.0075 mM NaH_2PO_4 (Balzergue et al., 2013). However, the concentration of 16:1ω5 in the roots indicated a higher AMF biomass content in plants under Low P, showing a positive response to mycorrhizal colonization. Therefore, the aggressiveness of mycorrhizal colonization in the root system did not correlate with our observations of negative or positive effects on plants in response to colonization by a single AMF species. In contrast, we observed that, despite the high colonization and a high accumulation of AMF biomass in the roots, mycorrhizal colonization can generate a positive response in *S. lycorpersicum*, which manifested itself as an increase in the area of the plant source leaves under Low P.

Although the inoculated plants under Null P and Low P showed statistically similar colonization patterns, the proportion of the average vesicles was higher in the root fragments of plants subjected to Low P, suggesting that AMF stores a higher proportion of C in intraradical structures under these conditions. In contrast, we observed the highest number of spores in the substrate of inoculated plants under Null P, which exhibited a negative response to mycorrhization. This suggests that under severe conditions of limited P availability, as in Null P, AMF partitions the received C predominantly toward extraradical resistance structures (Figure 6). Spores are the final C sinks of AMF, the structures where fungi mainly store lipids and glycogen to a

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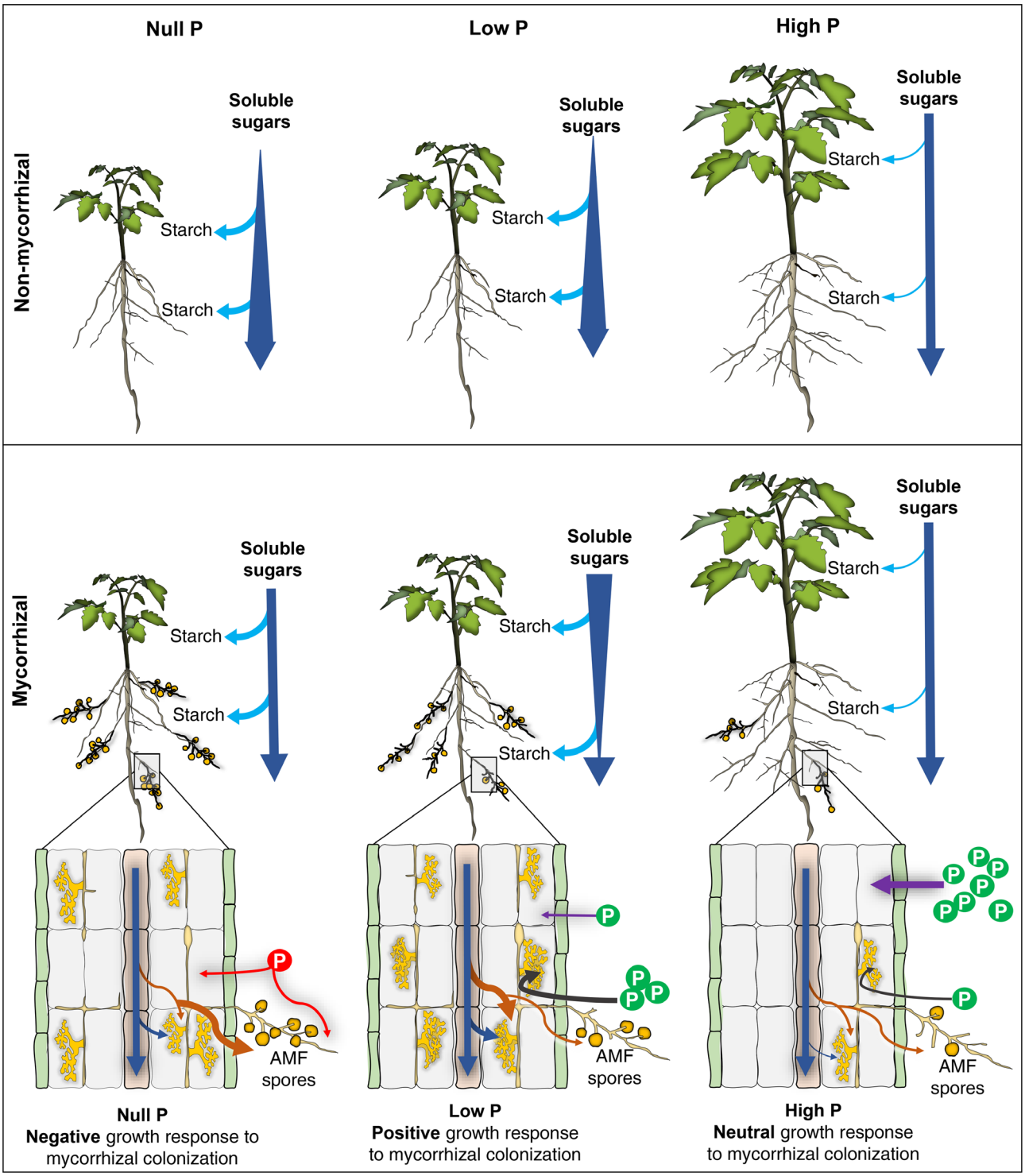

FIGURE 6 Legend on next page.

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lesser extent (Rich et al., 2017). In previous studies, in vitro sporulation of *R. irregularis* in monoxenic cultures was enhanced by reducing the concentration of P in the media. Likewise, in AMF colonized *Zea mays* under greenhouse conditions, spore formation was higher when P fertilization was omitted (Maitra et al., 2021). This supports the idea that C is preferentially partitioned to support AMF sporulation under limited P availability.

The overall increase in vesicles throughout the root system, and not only in root fragments, partially explains the increased accumulation of AMF biomass in Low P inoculated plants. Reduced sporulation was observed when positive growth responses to mycorrhizae were observed, suggesting that under such conditions, the C allocated to the AMF was predominantly used to build the intraradical hyphal network to support the bidirectional flow of nutrients between both symbionts (Figure 6). This idea is supported by the upregulation of the *SlPT4* gene observed in the AMF colonized roots of plants grown on Low P compared to plants grown on Null P. Overall, these results suggest that both increased intraradical C storage and an extended hyphal network are present when a host response is positive for mycorrhizal colonization.

Carbohydrates are a source of C transferred from plants to AMF; however, they are also required as precursors for FA biosynthesis (Ma et al., 2017). To support AMF lipid biosynthesis, 2-monoacylglycerol is first produced in the host plant and subsequently transferred to AMF for biomass accumulation and C storage (Jiang et al., 2017; Luginbuehl et al., 2017). Thus, according to our results, different plant responses to mycorrhizal colonization significantly influence C partitioning and allocation in AMF, supporting the idea that during mycorrhizal colonization, the fungal partner also senses the amount of C it receives from the plant (Kiers et al., 2011).

4.3 | Positive mycorrhizal response relates to higher photosynthetic potential

Solubility and composition of P sources have recently been reported to affect the interaction between tomatoes and *R. irregularis* (Andrino et al., 2021). Inoculated plants fertilized with a highly soluble P source (KH_2PO_4) showed a higher leaf area and photosynthetic rate than non-inoculated or inoculated plants fertilized with an organic P source (phytic acid) or with a low-solubility P source (apatite) (Andrino et al., 2021). These results suggest that the P provided as an orthophosphate is more efficiently absorbed from the substrate, assisted by AMF. Moreover, these results suggest that plants increase their photosynthetic area and C fixation rate to meet the C demands of AMF (Andrino et al., 2021). Our results on the increased area and soluble sugars of source leaves of *S. lycorpersicum* promoted by *R. irregularis* under Low P support this idea, suggesting that under these conditions, P is transferred to the plant by the AMF.

We also found higher SPAD values in SL1 from inoculated plants under Null and High P conditions, with a more pronounced difference in Null P, which was also observed in SL2 SPAD units from inoculated plants under Null P conditions. Increased SPAD unit values owing to mycorrhizal colonization have been previously observed across different plant species (Campanelli et al., 2013; Lopez de Andrade et al., 2015; Saia, Aissa, et al., 2020). There is a positive correlation between an increase in SPAD units and an improvement in photosynthetic rate (Reis et al., 2009). However, increased SPAD units have also been associated with impaired PSII and are considered an indicator of stress responses in flag leaves, which are the main source tissue in rice (Kumagai et al., 2009). We observed positive responses to mycorrhizal colonization as an increase in the photosynthetic area of source leaves at Low P, without significantly altering SPAD units, whereas inoculated plants grown under Null P and High P showed significant increases in SPAD units, even if the leaf area remained unchanged. These results suggest that when mycorrhizae promote growth responses under low P availability, the greater allocation of fixed C for the biosynthesis of soluble sugars in source leaves is the result of an increased photosynthetic leaf area, coupled with the attenuation of the stress response in photosynthetic tissues. In contrast, in negative or neutral plant growth responses, mycorrhizal symbiosis increases the chlorophyll content and potential photosynthetic capacity.

4.4 | P availability determines soluble sugars and starch partitioning and allocation in mycorrhizal plants

The increase of P in plant tissues may indirectly impact the concentration of soluble sugar in source leaves because P availability in

FIGURE 6 Effect of P availability and arbuscular mycorrhiza (AM) colonization on carbon flux and mycorrhizal development. In the absence of mycorrhizal colonization, plants accumulate soluble sugars in roots and increase the starch content in source leaves and roots as a response to P starvation. The negative, positive, or neutral plant growth responses to mycorrhizal colonization under three P availability conditions are related to different AM colonization performances, driven by different P acquisition conditions and modified patterns of carbon allocation and partitioning. Patterns of carbon allocation to starch or soluble sugar biosynthesis at High P are modified under Null P or Low P, increasing starch accumulation in source leaves and roots. The presence of AM symbiosis promotes the allocation of carbon to the soluble sugars biosynthesis in source tissues, regardless of the plant phenotypic response, while the allocation of carbohydrates and lipids to AMF is increased in plants showing a positive growth response. The development of mycorrhizal colonization also depends on the availability of P, showing greater performance under Low P conditions. The dark blue arrows indicate the allocation and partitioning of soluble sugars in source tissues and mycorrhizal roots; light blue arrows indicate carbon allocation to starch biosynthesis in source tissues and roots. Carbon partitioning and allocation in mycorrhizal roots are indicated using arrows as described at the bottom of the scheme. The differences in the thickness of the arrows are in agreement with the intensity of the nutrient fluxes between plant tissues or AMF structures. The Null P, Low P, and High P conditions represent Long Ashton's nutrient fertilization under three NaH_2PO_4 concentrations (0, 0.065, and 1.3 mM, respectively).

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mesophyll cells is essential for triose-P translocation from chloroplasts by the TRIOSE PHOSPHATE TRANSLOCATOR, an antiporter that exports triose-P in exchange for inorganic (Pi) from the cytoplasm. Chloroplast-imported Pi is further used in light-dependent stage reactions to regenerate ATP, whereas triose-P is used for soluble sugar biosynthesis (Flügge et al., 1989).

It has been previously reported that soluble sugar content and SPS activity in *Poncirus trifoliata* leaves increase in response to AM colonization by different AMF species (Wu et al., 2017). The in vivo irreversible reaction catalyzed by SPS activity is a rate-limiting step in Suc biosynthesis from photosynthesis-derived triose-P (Lunn & ap Rees, 1990), thus determining the amount of C that can be partitioned between plant organs. The overall increase in the ratio of total soluble sugar to starch in the source leaves of inoculated tomato plants in our study suggests that *R. irregularis* colonization promotes C partitioning for the biosynthesis of soluble sugars allocated to sink organs, regardless of the phenotypic response to mycorrhizal colonization.

Previous reports have shown that P deficiency upregulates AGPase activity, increasing starch content and releasing P from Calvin Cycle intermediates to ATP biosynthesis during photophosphorylation (MacNeill et al., 2017). Starch accumulation in the roots is the result of greater sink strength in conjunction with increased C partitioning to starch biosynthesis (Hammond & White, 2008). Here, we report that P starvation favors starch accumulation in source leaves and roots compared to High P conditions, while the starch content in inoculated and non-inoculated plants growing under the three P concentrations was statistically similar in source leaves or roots. The evaluation of *Lotus japonicus* mutants impaired in starch biosynthesis or degradation showed that root starch metabolism is not altered by mycorrhizal colonization, suggesting that the fungus absorbs most of the C in the form of soluble sugars transported from source tissues to the colonized roots (Gutjahr et al., 2011). Our results are congruent with this finding and indicate that starch metabolism in source leaves and roots is not significantly altered whether plants exhibit positive or negative growth responses to AM colonization.

4.5 | Enhancement of the sink capacity of roots in mycorrhizal plants

Soluble sugar accumulation in source leaves was observed in inoculated plants under all P concentrations, suggesting an increase in source strength exerted by mycorrhizal symbiosis. In contrast, a reduction in the content of soluble sugars in leaves has been previously described as a response to the increased mobilization of soluble sugars from source tissues to mycorrhizal roots (Doidy et al., 2012; Goddard et al., 2021). These differences could be related to factors that control the nutrient supply to the AMF, such as circadian regulation, light quality and intensity, plant age, and mycorrhizal development stage (Salmeron-Santiago et al., 2022).

The colonization of *S. lycorpersicum* by *F. mosseae* promotes the accumulation of Suc and fructose and reduces the Glc content in roots, suggesting an improvement in the sink capacity of roots colonized by AMF, and the reduction of Glc in roots points to the hexose uptake capacity of the AMF (Boldt et al., 2011). SUT transporters promote the translocation of Suc from source tissues to mycorrhizal roots for partitioning into the periarbuscular space, where Suc is hydrolyzed by cell wall-bound invertases to Glc and fructose (Bitterlich et al., 2014; Boldt et al., 2011; García-Rodríguez et al., 2007; Schaarschmidt et al., 2006). In roots, we observed that inoculated plants under Low P conditions showed a reduced content of total soluble sugars compared to non-inoculated plants, particularly in the fraction of total reducing sugars, which included Glc and total reducing ketoses. These results, together with the increase in the amount of 16:1ω5 quantified in inoculated plants grown under Low P conditions, suggest that positive growth responses to mycorrhiza are related to higher intraradical AMF biomass. Consequently, the host plant increases the allocation of C to AMF in the form of lipids to increase the intraradical hyphal network to support P uptake via the mycorrhizal pathway. This view supports the hypothesis that plants invest more C when they perceive a benefit in terms of P uptake from AMF (Kiers et al., 2011). Similar approaches comparing AMF species displaying positive or neutral phenotypes in *Glycine max* have explored the importance of sugar and lipid metabolism in such interactions and have proposed that positive growth responses in mycorrhizal plants are related to AMF with high colonization capabilities that enhance sugar mobilization and catabolism in mycorrhizal roots (Zhao et al., 2019).

Under Null P conditions, we observed upregulation of *SlLIN6* in inoculated plants, suggesting that at Null P, the unloading of Suc to the apoplast determines the sink strength in inoculated plants, and the mechanism is controlled by the hydrolysis of Suc in the apoplast by cell-wall bound invertases. The expression of *SlLIN6* has been localized in arbusculated cells, and its upregulation has been previously reported in mycorrhizal roots of tomato plants (García-Rodríguez et al., 2007; Schaarschmidt et al., 2006). Nevertheless, the overexpression of invertases in the roots of tobacco and *Medicago truncatula* at different subcellular localizations, including the apoplast, did not alter the mycorrhizal development observed in non-overexpressing genotypes, despite the increase in the content of hexoses in roots (Schaarschmidt et al., 2007). Instead, it has been suggested that other mechanisms, such as phosphate supply and plant defense responses, rather than hexose supply to AMF by invertase activity, are more likely to regulate mycorrhizal colonization (Schaarschmidt et al., 2007). This is congruent with our observations that the upregulation of *SlLIN6* did not promote the increase in AMF biomass to the highest level under the conditions we tested. In addition, although mycorrhizal colonization caused a significant reduction in total ketoses, it did not cause a significant reduction in the accumulation of total soluble sugars. These results suggest that total soluble sugars accumulate at a similar rate in inoculated and non-inoculated plants under limited P availability. Therefore, the supply of hexose does not limit mycorrhizal development.

Recently, it was reported that the upregulation of a cell wall-bound invertase gene in potato occurs in parallel with the upregulation of genes coding for SWEET, which are responsible for the

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mobilization of Suc and monosaccharides (Manck-Götzenberger & Requena, 2016). In addition, it has been reported that the constitutive overexpression of *StSWEET7a*, a Glc and fructose transporter from potato, induces accumulation of *StInvCD141* transcripts in non-mycorrhizal roots and increases the frequency and abundance of mycorrhizal colonization when plants are inoculated with *R. irregularis* (Tamayo et al., 2022). It was proposed that the overexpression of *StSWEET7a* depletes the apoplast from monosaccharides, and consequently, the sink strength is increased by the upregulation of cell wall-bound invertases to promote the unloading of Suc to the apoplast (Tamayo et al., 2022). In tomato, Suc depletion in the periarbuscular space mediated by the SUT2 transporter has been reported as a mechanism to control the supply of C to the AMF. Silencing *SlSUT2* abolishes the positive growth response to AM symbiosis and increases root colonization (Bitterlich et al., 2014). In *M. truncatula*, the increased expression of SUT transporter family members under low P conditions (NaH_2PO_4, 0.13 mM) was even greater under *Rhizophagus* sp. colonization (Doidy et al., 2012). These findings suggest that both P-limiting conditions and the presence of AMF in roots promote SUT-mediated mechanisms to regulate C allocation to AMF. We hypothesize that under extreme P starvation, Null P conditions, sugars (Suc and monosaccharides) are depleted from the apoplast, and mycorrhizal colonization induces root sink strength by the upregulation of cell wall-bound invertases. As previously mentioned, plants that showed a negative growth response to mycorrhizal colonization had similar total soluble sugar content in their roots as non-inoculated plants. This result suggests that C may not be the limiting factor in the negative response to AM colonization; instead, P deficiency could be the main driver of a negative plant phenotypic outcome (Figure 6). In addition, the lower development of intraradical AMF biomass as revealed by the accumulation of the lipid 16:1ω5 and the lower accumulation of *SlPT4* transcripts compared to inoculated plants grown on Low P, supports the idea that the C transferred to the AMF depends on the "reward" received from the AMF (van't Padje et al., 2021). Other non-described mechanisms that regulate lipid biosynthesis and transfer to the AMF could operate under P starvation conditions to limit the intraradical development of AMF.

Under Low P conditions, we observed an upregulation of *SlSUS1* in inoculated plants, whereas the expression of *SlLIN6* remained at the same level in the roots of inoculated and non-inoculated plants. Silencing of *MtSuc1*, a Suc synthase-encoding gene that is upregulated in mycorrhizal roots in *M. truncatula*, led to a reduction in mycorrhizal colonization and the formation of non-fully developed and early senescent arbuscules (Baier et al., 2010). Suc catabolism by SuSY is important during the establishment and maintenance of mycorrhizal symbiosis (Baier et al., 2010). *SlSUS1* has been shown to be upregulated in tomato plants and it has been thought to support the metabolic activity of mycorrhizal roots rather than to provide hexoses to AMF (García-Rodríguez et al., 2007). Instead, our results suggest that, in mycorrhizal roots, Suc is catabolized by SuSY in the cytoplasm of colonized root cells to increase the allocation of C to the biosynthesis of lipids when plants show a positive growth response from mycorrhizal symbiosis. Based on these findings, we propose that sink strength in plants showing positive growth responses is controlled by Suc catabolism mediated by SUS.

The activity of SuSY is related to the biosynthesis of starch and cellulose, but it is also known that its activity is required for the canalization of C to plastids for lipid biosynthesis (Núñez et al., 2008; Stein & Granot, 2019). It has been reported that P starvation reconfigures the lipidome of tomato plants, and molecular evidence from the non-mycorrhizal model plant *Arabidopsis thaliana* has demonstrated that the expression of lysophosphatidic acid phosphatase (LPA phosphatase), an enzyme that catalyzes the biosynthesis of monoacylglycerols from LPA, is upregulated during P starvation (Pfaff et al., 2020; Reddy et al., 2010). The reconfiguration of the lipidome in *A. thaliana* is regulated by *PHOSPHATE STARVATION RESPONSE 1* (*PHR1*), a transcription factor acting as a master regulator of a plant's response to limited P availability (Pant et al., 2015). The knockout mutant of *PHR1* in *A. thaliana* accumulates less soluble sugars and starch under P-starving conditions (Nilsson et al., 2007). Recently, using rice and tomato plants, it has been shown that members of the PHR transcription family and their interaction with their negative regulator, SPX1, are responsible for controlling mycorrhizal development in response to P starvation (Das et al., 2022; Liao et al., 2022). The silencing of *PHR2* dramatically reduces AMF colonization in rice and compromises the expression of genes required for precontact and karrikin signaling, strigolactone and apocarotenoid biosynthesis, lipid and P exchange and signal transduction, and the regulation of colonization (Das et al., 2022). In tomato plants, *SlSPX1* silencing (SYG1 [suppressor of yeast GPA1]/Pho81[phosphate 81]/XPR1 [xenotropic and polytropic retrovirus receptor 1]), a negative regulator of PHR transcription factors, induces mycorrhizal colonization even when P is sufficient, proving that *SlSPX1* is a major repressor of mycorrhizal symbiosis when P is not limited (Liao et al., 2022). These findings suggest that C partitioning and allocation in P-starving conditions are also regulated by the P-starving response mediated by PHR transcription factors. Together with our observations of tomato plants under Low P, these findings also support the possibility that small amounts of P are sufficient to allocate a higher amount of C to the biosynthesis of specific lipids transferred to the AMF to increase P uptake by the mycorrhizal pathway, which might be controlled by PHR transcription factors.

Suc and monosaccharide depletion in the periarbuscular space, mediated by the action of SUT and SWEET transporters, seems to be relevant in regulating mycorrhizal symbiosis development (Bitterlich et al., 2014; Doidy et al., 2012; Tamayo et al., 2022). According to our results, limited P availability seems to induce a sugar reduction in the periarbuscular space. Under such conditions, the AMF increases the sink strength by the upregulation of cell-wall bound invertases. Small amounts of P appear to be sufficient to shift C allocation to lipid biosynthesis to increase intraradical AMF biomass and improve P uptake by the mycorrhizal pathway.

5 | CONCLUSION

The scheme shown in Figure 6 integrates the findings and conclusions. Consistent with our results, we hypothesize that AM colonization enhances sink strength even at low levels of colonization.

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Moreover, the C allocated to starch biosynthesis in source tissues promoted by P deficiency under Null and Low P conditions does not limit the source strength stimulation exerted by mycorrhizal colonization. The increased source strength provided a greater concentration of soluble sugars delivered to the mycorrhizal sinks. Simultaneously, sink strength was enhanced in the mycorrhizal roots in the presence of AMF.

The accumulation of soluble sugars in the roots of AMF inoculated plants under P-deficient conditions is a consequence of the increased sink capacity of the roots, suppressing plant growth. However, the host plant restricts the C reward to the AMF because of the Low P provided during symbiosis, limiting the development of mycorrhiza. Resource restriction of AMF would thus promote spore formation.

A positive growth response to AM colonization requires the host plant to invest a higher proportion of carbohydrates and lipids to supply AMF. Under such conditions, the host plant allocates a higher proportion of soluble sugars to the biosynthesis of 2-monoacylglycerol, which supports 16:1ω5 biosynthesis and, subsequently, biomass production and C storage in AMF. Consequently, this led to a reduction in the content of soluble sugars in the mycorrhizal roots. AMF inoculated plants growing under Low P conditions would take up P via the mycorrhizal pathway; thus, C taken up by AMF would be preferentially allocated to biomass synthesis to maintain the bidirectional nutrient flux, while the C allocated to spore formation would be reduced.

This suggests that positive or negative plant growth responses to mycorrhizal colonization are a consequence of C partitioning among tissues and allocation to different metabolic pathways in the host, influenced by AMF. Further studies on gene expression and enzymatic activities of molecular elements regulating C partitioning and allocation in plants will reveal the host-imposed molecular mechanisms that regulate the amount of C allocated to sustain AMF demand in mycorrhizal symbiosis.

AUTHOR CONTRIBUTIONS

Ana T. Chávez-Bárcenas, Isaac A. Salmeron-Santiago, and Miguel Martínez-Trujillo conceived the idea. Ana T. Chávez-Bárcenas and Isaac A. Salmeron-Santiago performed the experiments. John Larsen conducted the lipid quantification analysis experiments. Isaac A. Salmeron-Santiago and Ana T. Chávez-Bárcenas writing—original draft preparation. Ana T. Chávez-Bárcenas, Juan J. Valdez-Alarcón, and Miguel Martínez-Trujillo funding acquisition. All authors participated in data analysis, writing review, and editing. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

The authors wish to acknowledge I. E. Maldonado-Mendoza and M. López-Meyer for the kind donation of the in vitro monoxenic cultured spores of *R. irregularis* (DAOM 197198), which we propagated to generate the inoculum used in this work. I. A. S.-S. received a scholarship (732373) granted by the Consejo Nacional de Ciencia y Tecnonogía (CONACYT). To conduct this research, A. T. C.-B. provided funding from Coordinación de la Investigación Científica (Universidad Michoacana de San Nicolás de Hidalgo), project (5872608).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

An, J., Zeng, T., Ji, C., de Graaf, S., Zheng, Z., Xiao, T.T. et al. (2019) A *Medicago truncatula* SWEET transporter implicated in arbuscule maintenance during arbuscular mycorrhizal symbiosis. *The New Phytologist*, 224, 396–408.

Andrino, A., Guggenberger, G., Sauheitl, L., Burkart, S. & Boy, J. (2021) Carbon investment into mobilization of mineral and organic phosphorus by arbuscular mycorrhiza. *Biology and Fertility of Soils*, 57, 47–64.

Baier, M.C., Keck, M., Gödde, V., Niehaus, K., Küster, H. & Hohnjec, N. (2010) Knockdown of the symbiotic sucrose synthase *MtSucS1* affects arbuscule maturation and maintenance in mycorrhizal roots of *Medicago truncatula*. *Plant Physiology*, 152, 1000–1014.

Balzergue, C., Chabaud, M., Barker, D.G., Bécard, G. & Rochange, S.F. (2013) High phosphate reduces host ability to develop arbuscular mycorrhizal symbiosis without affecting root calcium spiking responses to the fungus. *Frontiers in Plant Science*, 4, 426.

Begum, N., Qin, C., Ahanger, M.A., Raza, S., Khan, M.I., Ashraf, M. et al. (2019) Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. *Frontiers in Plant Science*, 10, 1068.

Bihmidine, S., Hunter, C.T., Johns, C.E., Koch, K.E. & Braun, D.M. (2013) Regulation of assimilate import into sink organs: update on molecular drivers of sink strength. *Frontiers in Plant Science*, 4, 177.

Bitterlich, M., Krügel, U., Boldt-Burisch, K., Franken, P. & Kühn, C. (2014) The sucrose transporter SlSUT2 from tomato interacts with brassinosteroid functioning and affects arbuscular mycorrhiza formation. *The Plant Journal*, 78, 877–889.

Boldt, K., Pörs, Y., Haupt, B., Bitterlich, M., Kühn, C., Grimm, B. et al. (2011) Photochemical processes, carbon assimilation and RNA accumulation of sucrose transporter genes in tomato arbuscular mycorrhiza. *Journal of Plant Physiology*, 168, 1256–1263.

Braun, D.M. (2012) SWEET! The pathway is complete. *Science*, 335, 173–174.

Campanelli, A., Ruta, C., De, M.G. & Morone-Fortunato, I. (2013) The role of arbuscular mycorrhizal fungi in alleviating salt stress in *Medicago sativa* L. var. icon. *Symbiosis*, 59, 65–76.

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, 601–610.

13993054, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/ppl.13857 by Cstc Organización Central Om (Oficialia Mayor) (Unci), Wiley Online Library on [25/02/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Chen, A., Gu, M., Wang, S., Chen, J. & Xu, G. (2018) Transport properties and regulatory roles of nitrogen in arbuscular mycorrhizal symbiosis. *Seminars in Cell & Developmental Biology*, 74, 80–88.

Chen, S., Hajirezaei, M. & Börnke, F. (2005) Differential expression of sucrose-phosphate synthase isoenzymes in tobacco reflects their functional specialization during dark-governed starch mobilization in source leaves. *Plant Physiology*, 139, 1163–1174.

Chiu, C.H. & Paszkowski, U. (2019) Mechanisms and impact of symbiotic phosphate acquisition. *Cold Spring Harbor Perspectives in Biology*, 11, a034603.

Cope, K.R., Kafle, A., Yakha, J.K., Pfeffer, P.E., Strahan, G.D., Garcia, K. et al. (2022) Physiological and transcriptomic response of *Medicago truncatula* to colonization by high- or low-benefit arbuscular mycorrhizal fungi. *Mycorrhiza*, 32, 281–303.

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, 477.

Doidy, J., Van Tuinen, D., Lamotte, O., Corneillat, M., Alcaraz, G. & Wipf, D. (2012) The *Medicago truncatula* sucrose transporter family: characterization and implication of key members in carbon partitioning towards arbuscular mycorrhizal fungi. *Molecular Plant*, 5, 1346–1358.

DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28, 350–356.

Ferrol, N., Azcón-Aguilar, C. & Pérez-Tienda, J. (2019) Review: Arbuscular mycorrhizas as key players in sustainable plant phosphorus acquisition: an overview on the mechanisms involved. *Plant Science*, 280, 441–447.

Flügge, U.I., Fischer, K., Gross, A., Sebald, W., Lottspeich, F. & Eckerskorn, C. (1989) The triose phosphate-3-phosphoglycerate-phosphate translocator from spinach chloroplasts: nucleotide sequence of a full-length cDNA clone and import of the in vitro synthesized precursor protein into chloroplasts. *The EMBO Journal*, 8, 39–46.

García-Rodríguez, S., Azcón-Aguilar, C. & Ferrol, N. (2007) Transcriptional regulation of host enzymes involved in the cleavage of sucrose during arbuscular mycorrhizal symbiosis. *Physiologia Plantarum*, 129, 737–746.

Genre, A., Lanfranco, L., Perotto, S. & Bonfante, P. (2020) Unique and common traits in mycorrhizal symbioses. *Nature Reviews. Microbiology*, 18, 649–660.

Gerdemann, J.W. & Nicolson, T.H. (1963) Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society*, 46, 235–244.

Goddard, M.L., Belval, L., Martin, I.R., Roth, L., Laloue, H., Deglène-Benbrahim, L. et al. (2021) Arbuscular mycorrhizal symbiosis triggers major changes in primary metabolism together with modification of defense responses and signaling in both roots and leaves of *Vitis vinifera*. *Frontiers in Plant Science*, 12, 721614.

Graham, J.H. & Abbott, L.K. (2000) Wheat responses to aggressive and non-aggressive arbuscular mycorrhizal fungi. *Plant and Soil*, 220, 207–218.

Gutjahr, C. & Parniske, M. (2013) Cell and developmental biology of arbuscular mycorrhiza symbiosis. *Annual Review of Cell and Developmental Biology*, 29, 593–617.

Gutjahr, C., Novero, M., Welham, T., Wang, T. & Bonfante, P. (2011) Root starch accumulation in response to arbuscular mycorrhizal colonization differs among *Lotus japonicus* starch mutants. *Planta*, 234, 639–646.

Hammond, J.P. & White, P.J. (2008) Sucrose transport in the phloem: integrating root responses to phosphorus starvation. *Journal of Experimental Botany*, 59, 93–109.

Hart, M.M. & Reader, R.J. (2002) Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *The New Phytologist*, 153, 335–344.

Hartmann, H., Bahn, M., Carbone, M. & Richardson, A.D. (2020) Plant carbon allocation in a changing world—challenges and progress: Introduction to a virtual issue on carbon allocation. *The New Phytologist*, 227, 981–988.

Hatchel, G.E., Berry, C.R. & Muse, H.D. (1985) Nondestructive indices related to aboveground biomass of young loblolly and sand pines on ectomycorrhizal and fertilizer plots. *Forest Science*, 31, 419–427.

Hennion, N., Durand, M., Vriet, C., Doidy, J., Maurousset, L., Lemoine, R. et al. (2019) Sugars en route to the roots. Transport, metabolism and storage within plant roots and towards microorganisms of the rhizosphere. *Physiologia Plantarum*, 165, 44–57.

Ivanov, S., Austin, J., Berg, R.H. & Harrison, M.J. (2019) Extensive membrane systems at the host–arbuscular mycorrhizal fungus interface. *Nature Plants*, 5, 194–203.

Janos, D.P. (2007) Plant responsiveness to mycorrhizas differs from dependence upon mycorrhizas. *Mycorrhiza*, 17, 75–91.

Jiang, Y., Wang, W., Xie, Q., Liu, N., Liu, L., Wang, D. et al. (2017) Plants transfer lipids to sustain colonization by mutualistic mycorrhizal and parasitic fungi. *Science*, 356, 1172–1175.

Jung, S.C., Martinez-Medina, A., Lopez-Raez, J.A. & Pozo, M.J. (2012) Mycorrhiza-induced resistance and priming of plant defenses. *Journal of Chemical Ecology*, 38, 651–664.

Kakouridis, A., Hagen, J.A., Kan, M.P., Mambelli, S., Feldman, L.J., Herman, D.J. et al. (2022) Routes to roots: direct evidence of water transport by arbuscular mycorrhizal fungi to host plants. *The New Phytologist*, 236, 210–221.

Kaschuk, G., Kuyper, T.W., Leffelaar, P.A., Hungria, M. & Giller, K.E. (2009) Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses? *Soil Biology and Biochemistry*, 41, 1233–1244.

Kerr, P.S. & Huber, S.C. (1987) Coordinate control of sucrose formation in soybean leaves by sucrose-phosphate synthase and fructose-2,-6-bisphosphate. *Planta*, 170, 197–204.

Kiers, E.T., Duhamel, M., Beesetty, Y., Mensah, J.A., Franken, O., Verbruggen, E. et al. (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science*, 333, 880–882.

Koch, K. (2004) Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Current Opinion in Plant Biology*, 7, 235–246.

Koch, K.E., Wu, Y. & Xu, J. (1996) Sugar and metabolic regulation of genes for sucrose metabolism: potential influence of maize sucrose synthase and soluble invertase responses on carbon partitioning and sugar sensing. *Journal of Experimental Botany*, 47, 1179–1185.

Kumagai, E., Araki, T. & Kubota, F. (2009) Correlation of chlorophyll meter readings with gas exchange and chlorophyll fluorescence in flag leaves of rice (*Oryza sativa* L.) plants. *Plant Production Science*, 12, 50–53.

Kühn, C. & Grof, C.P.L. (2010) Sucrose transporters of higher plants. *Current Opinion in Plant Biology*, 13, 287–297.

Liao, D., Sun, C., Liang, H., Wang, Y., Bian, X., Dong, C. et al. (2022) SlSPX1-SlPHR complexes mediate the suppression of arbuscular mycorrhizal symbiosis by phosphate repletion in tomato. *Plant Cell*, 34, 4045–4065.

Lim, G.H., Singhal, R., Kachroo, A. & Kachroo, P. (2017) Fatty acid- and lipid-mediated signaling in plant defense. *Annual Review of Phytopathology*, 55, 505–536.

Liu, J., Liu, J., Liu, J., Cui, M., Huang, Y., Tian, Y. et al. (2019) The potassium transporter SlHAK10 is involved in mycorrhizal potassium uptake. *Plant Physiology*, 180, 465–479.

Livak, K.J. & Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, 25, 402–408.

Lopez de Andrade, S.A., Domingues, A.P. & Mazzafera, P. (2015) Photosynthesis is induced in rice plants that associate with arbuscular mycorrhizal fungi and are grown under arsenate and arsenite stress. *Chemosphere*, 134, 141–149.

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Luginbuehl, L.H., Menard, G.N., Kurup, S., Van Erp, H., Radhakrishnan, G. V., Breakspear, A. et al. (2017) Fatty acids in arbuscular mycorrhizal fungi are synthesized by the host plant. *Science*, 356, 1175–1178.

Lunn, J.E. & ap Rees, T. (1990) Apparent equilibrium constant and mass-action ratio for sucrose-phosphate synthase in seeds of *Pisum sativum*. *The Biochemical Journal*, 267, 739–743.

Lü, L.-H., Zou, Y.-N. & Wu, Q.-S. (2018) Relationship between arbuscular mycorrhizas and plant growth: improvement or depression? In: Giri, B., Prasad, R. & Varma, A. (Eds.) *Root biology*. Cham: Elsevier, pp. 451–464.

Ma, Y., Yin, Z. & Ye, J. (2017) Lipid biosynthesis and regulation in *Jatropha*, an emerging model for woody energy plants. In: Tsuchimoto, S. (Ed.) *The Jatropha genome. Compendium of plant genomes*. Cham: Springer, pp. 113–127.

MacNeill, G.J., Mehrpouyan, S., Minow, M.A.A., Patterson, J.A., Tetlow, I. J. & Emes, M.J. (2017) Starch as a source, starch as a sink: the bifunctional role of starch in carbon allocation. *Journal of Experimental Botany*, 68, 4433–4453.

Maitra, P., Al-Rashid, J., Barman, N.C., Khan, M.N.M., Mandal, D., Rasul, N. M. et al. (2021) Sand particle size and phosphorus amount affect *Rhizophagus irregularis* spore production using in vitro propagated spore as a starter inoculum in rhizosphere of maize (*Zea mays*) plantlets. *Journal of Fungi*, 7, 846.

Maldonado-Mendoza, I.E. & Harrison, M.J. (2018) *RiArsB* and *RiMT-11*: Two novel genes induced by arsenate in arbuscular mycorrhiza. *Fungal Biology*, 122, 121–130.

Manck-Götzenberger, J. & Requena, N. (2016) Arbuscular mycorrhiza symbiosis induces a major transcriptional reprogramming of the potato SWEET sugar transporter family. *Frontiers in Plant Science*, 7, 487.

Nelson, N. (1944) A photometric adaptation of the somogyi method for the determination of glucose. *The Journal of Biological Chemistry*, 153, 375–380.

Nilsson, L., Müller, R. & Nielsen, T.H. (2007) Increased expression of the MYB-related transcription factor, *PHR1*, leads to enhanced phosphate uptake in *Arabidopsis thaliana*. *Plant, Cell and Environment*, 30, 1499–1512.

Nouri, E., Breuillin-Sessoms, F., Feller, U. & Reinhardt, D. (2014) Phosphorus and nitrogen regulate arbuscular mycorrhizal symbiosis in *Petunia hybrida*. *PLoS One*, 9, e90841.

Núñez, J.G.A., Kronenberger, J., Wuillème, S., Lepiniec, L. & Rochat, C. (2008) Study of AtSUS2 localization in seeds reveals a strong association with plastids. *Plant & Cell Physiology*, 49, 1621–1626.

Pant, B.D., Burgos, A., Pant, P., Cuadros-Inostroza, A., Willmitzer, L. & Scheible, W.R. (2015) The transcription factor PHR1 regulates lipid remodeling and triacylglycerol accumulation in *Arabidopsis thaliana* during phosphorus starvation. *Journal of Experimental Botany*, 66, 1907–1918.

Pfaff, J., Denton, A.K., Usadel, B. & Pfaff, C. (2020) Phosphate starvation causes different stress responses in the lipid metabolism of tomato leaves and roots. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, 1865, 158763.

Phillips, J.M. & Hayman, D.S. (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, 55, 158–161.

Poorter, H. & Nagel, O. (2000) The role of biomass allocation in the growth response of plants to different levels of light, CO2, nutrients and water: a quantitative review. *Australian Journal of Plant Physiology*, 27, 595–607.

Poorter, H. & Pothmann, P. (1992) Growth and carbon economy of a fast-growing and a slow-growing grass species as dependent on ontogeny. *The New Phytologist*, 120, 159–166.

Reddy, V.S., Rao, D.K.V. & Rajasekharan, R. (2010) Functional characterization of lysophosphatidic acid phosphatase from *Arabidopsis thaliana*. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, 1801, 455–461.

Reis, A.R., Favarin, J.L., Malavolta, E., Júnior, J.L. & Moraes, M.F. (2009) Photosynthesis, chlorophylls, and SPAD readings in coffee leaves in relation to nitrogen supply. *Communications in Soil Science and Plant Analysis*, 40, 1512–1528.

Reuscher, S., Akiyama, M., Yasuda, T., Makino, H., Aoki, K., Shibata, D. et al. (2014) The sugar transporter inventory of tomato: genome-wide identification and expression analysis. *Plant & Cell Physiology*, 55, 1123–1141.

Rich, M.K., Nouri, E., Courty, P.E. & Reinhardt, D. (2017) Diet of arbuscular mycorrhizal fungi: bread and butter? *Trends in Plant Science*, 22, 652–660.

Roe, J.H. (1934) A colorimetric method for the determination of fructose in blood and urine. *The Journal of Biological Chemistry*, 107, 15–22.

Ruytinx, J., Kafle, A., Usman, M., Coninx, L., Zimmermann, S.D. & Garcia, K. (2020) Micronutrient transport in mycorrhizal symbiosis; zinc steals the show. *Fungal Biology Reviews*, 34, 1–9.

Saia, S., Aissa, E., Luziatelli, F., Ruzzi, M., Colla, G., Ficca, A.G. et al. (2020) Growth-promoting bacteria and arbuscular mycorrhizal fungi differentially benefit tomato and corn depending upon the supplied form of phosphorus. *Mycorrhiza*, 30, 133–147.

Saia, S., Tamayo, E., Schillaci, C. & De Vita, P. (2020) Arbuscular mycorrhizal fungi and nutrient cycling in cropping systems. In: Datta, R., Meena, R., Pathan, S. & Ceccherini, M. (Eds.) *Carbon and nitrogen cycling in soil*. Singapore: Springer Singapore, pp. 87–115.

Salmeron-Santiago, I.A., Martínez-Trujillo, M., Valdez-Alarcón, J.J., Pedraza-Santos, M.E., Santoyo, G., Pozo, M.J. et al. (2022) An updated review on the modulation of carbon partitioning and allocation in arbuscular mycorrhizal plants. *Microorganisms*, 10, 75.

Sasser, M. (1990). Technical Note 101. MIDI, Inc. https://doi.org/10.13140/RG.2.1.1883.4805

Schaarschmidt, S., Kopka, J., Ludwig-mu, J. & Hause, B. (2007) Regulation of arbuscular mycorrhization by apoplastic invertases: enhanced invertase activity in the leaf apoplast affects the symbiotic interaction. *The Plant Journal*, 51, 390–405.

Schaarschmidt, S., Roitsch, T. & Hause, B. (2006) Arbuscular mycorrhiza induces gene expression of the apoplastic invertase LIN6 in tomato (*Lycopersicon esculentum*) roots. *Journal of Experimental Biology*, 57, 4015–4023.

Schneider, C.A., Rasband, W.S. & Eliceiri, K.W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9, 671–675.

Smith, A.M. & Zeeman, S.C. (2006) Quantification of starch in plant tissues. *Nature Protocols*, 1, 1342–1345.

Smith, G.S., Johnston, C.M. & Cornforth, I.S. (1983) Comparison of nutrient solutions for growth of plants in sand culture. *The New Phytologist*, 94, 537–548.

Smith, S.E., Jakobsen, I., Grønlund, M. & Smith, F.A. (2011) Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiology*, 156, 1050–1057.

Somogyi, M. (1952) Notes on sugar determination. *The Journal of Biological Chemistry*, 195, 19–23.

Spatafora, J.W., Chang, Y., Benny, G.L., Lazarus, K., Smith, M.E., Berbee, M. L. et al. (2016) A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia*, 108, 1028–1046.

Stein, O. & Granot, D. (2019) An overview of sucrose synthases in plants. *Frontiers in Plant Science*, 10, 95.

Tamayo, E., Figueira-Galán, D., Manck-Götzenberger, J. & Requena, N. (2022) Overexpression of the potato monosaccharide transporter *StSWEET7a* promotes root colonization by symbiotic and pathogenic fungi by increasing root sink strength. *Frontiers in Plant Science*, 13, 837231.

13993054, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/ppl.13857 by Cstc Organización Central Om (Oficialia Mayor) (Utrc), Wiley Online Library on [25/02/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Thirkell, T.J., Grimmer, M., James, L., Pastok, D., Allary, T., Elliott, A. et al. (2022) Variation in mycorrhizal growth response among a spring wheat mapping population shows potential to breed for symbiotic benefit. *Food and Energy Security*, 11, e370.

Tran, B.T.T., Watts-Williams, S.J. & Cavagnaro, T.R. (2019) Impact of an arbuscular mycorrhizal fungus on the growth and nutrition of fifteen crop and pasture plant species. *Functional Plant Biology*, 46, 732–742.

Trouvelot, A., Kough, J.L. & Gianinazzi-Pearson, V. (1896) Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. In: Gianinazzi-Pearson, V. & Gianinazzi, S. (Eds.) *Physiological and genetical aspects of mycorrhizae*. Paris, France: INRA Press, pp. 217–221.

van't Padje, A., Werner, G.D.A. & Kiers, E.T. (2021) Mycorrhizal fungi control phosphorus value in trade symbiosis with host roots when exposed to abrupt 'crashes' and 'booms' of resource availability. *The New Phytologist*, 229, 2933–2944.

Wang, S., Chen, A., Xie, K., Yang, X., Luo, Z., Chen, J. et al. (2020) Functional analysis of the OsNPF4.5 nitrate transporter reveals a conserved mycorrhizal pathway of nitrogen acquisition in plants. *Proceedings of the National Academy of Sciences of the United States of America*, 117, 16649–16659.

Werner, G.D.A. & Kiers, E.T. (2015) Order of arrival structures arbuscular mycorrhizal colonization of plants. *The New Phytologist*, 205, 1515–1524.

Wipf, D., Krajinski, F., van Tuinen, D., Recorbet, G. & Courty, P.E. (2019) Trading on the arbuscular mycorrhiza market: from arbuscules to common mycorrhizal networks. *The New Phytologist*, 223, 1127–1142.

Wu, H.-H., Zou, Y.-N., Rahman, M.M., Ni, Q.-D. & Wu, Q.-S. (2017) Mycorrhizas alter sucrose and proline metabolism in trifoliate orange exposed to drought stress. *Scientific Reports*, 7, 42389.

Xu, G.H., Chague, V., Melamed-Bessudo, C., Kapulnik, Y., Jain, A., Raghothama, K.G. et al. (2007) Functional characterization of LePT4: a phosphate transporter in tomato with mycorrhiza-enhanced expression. *Journal of Experimental Botany*, 58, 2491–2501.

Zhao, S., Chen, A., Chen, C., Li, C., Xia, R. & Wang, X. (2019) Transcriptomic analysis reveals the possible roles of sugar metabolism and export for positive mycorrhizal growth responses in soybean. *Physiologia Plantarum*, 166, 712–728.

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: Salmeron-Santiago, I.A., Martínez-Trujillo, M., Valdez-Alarcón, J.J., Pedraza-Santos, M.E., Santoyo, G., López, P.A. et al. (2023) Carbohydrate and lipid balances in the positive plant phenotypic response to arbuscular mycorrhiza: increase in sink strength. *Physiologia Plantarum*, 175(1), e13857. Available from: https://doi.org/10.1111/ppl.13857

13993054, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/ppl.13857 by Cstc Organización Central Om (Oficialia Mayor) (Unci), Wiley Online Library on [25/02/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License