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Interactions between Root Hair Development and Arbuscular Mycorrhizal Fungal Colonization in Trifoliate Orange Seedlings in Response to P Levels

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Abstract: Both arbuscular mycorrhizal (AM) fungi and root hairs are crucial in facilitating plant uptake of phosphorus (P), while it is unclear whether and how they respond to varying P supplies. In order to explore how AM fungal colonization and root hair development are affected by substrate P supply, trifoliate orange (*Poncirus trifoliata*) seedlings were inoculated with AM fungus *Rhizophagus intraradices* and grown under low, moderate, and high P conditions; then, root hair morphological features and AM fungal colonization were measured. Following 120 days of AM fungal inoculation, root hair density, root hair length, AM fungal colonization rate, arbuscule colonization rate, and AM fungal colonization frequency all increased significantly under P-deficient conditions but decreased under high P conditions. Moreover, the colonization of AM fungi had a major impact on root hair formation by altering the expression of related genes and the growth of epidermal cells. The effect of AM fungi was dependent on P supply levels, as evidenced by the fact that root hair density and length increased at high P levels but decreased at low P levels. As a result, root hairs may serve as a preferential site for AM fungal colonization, and their morphology could influence the early stage of AM symbiosis establishment.

Keywords: arbuscular mycorrhiza; epidermis cell development; phosphorus; root hair; wheatgerm agglutinin

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1. Introduction

Phosphorus (P) is an essential macronutrient required for the growth and development of plants. It is absorbed by plant roots from the soil in the form of orthophosphate (ortho-P) [1,2]. However, crops often absorb less than 1% of ortho-P in the soil solution, resulting in sub-optimal crop productivity [3]. Plants have developed a range of strategies to adapt to a lack of P and enhance their ability to obtain P [4,5]. Plants can boost their P-uptake efficiency by developing numerous root hairs and forming arbuscular mycorrhizal (AM) associations [6].

Several crops, including wheat, citrus, beans, and tomato, rely heavily on root hairs for P absorption. Under the condition of P deficit, root hairs can account for up to 90% of total P uptake [7]. However, root hairs have been proven to be unnecessary when there is an adequate supply of P that does not limit plant growth [8,9]. Longer root hairs in plants are more effective at absorbing P, leading to a considerable increase in biomass production [10]. AM symbiosis is thought to be greatly important for increasing the ability of the host plant to increase P acquisition [11]. AM plants have the potential to uptake P via epidermal cells,

root hairs, and external AMF hyphae in the soil [12]. Actually, plants have high affinity P transporters within the epidermis, and P transporters of AM fungi are located in the external hyphae [13]. Root hair development and AM system establishment are influenced by environmental and genetic factors, including P levels in substrates [14]. Low P levels stimulate root hair formation and elongation while also improving the establishment of AM symbiosis [15,16].

Transcriptional regulators such as GL2, TTG, and WER play a key role in deciding the fate of root hair cells and stimulating their growth. Mutations in these genes can lead to the formation of root hairs instead of non-hair cells [17–20]. Conversely, the CPC and TRY are essential for determining the fate of hair cells, and the mutations in these genes can result in the formation of non-hair cells [21–23]. Moreover, genes like *ROPs*, *LRX1*, and *FER* are required for initiating root hair growth and tip elongation, which has a substantial impact on the initial phase of root hair development [24–26].

The AM and nodule systems are examples of a common plant–microbe symbiosis that has resulted in adaptations in both plants and microbionts. These systems also share key components of signaling pathways that are required for initiating their symbiotic program [27]. The common symbiosis signaling pathway (CSSP) consists of a set of shared genes, including DMI1/POLLUX and CASTOR [28], DMI2/SYMRK, DMI3/CcaMK [29], NUP85, NUP133 [30,31], CYCLOPS/IPD3, and NANE [32,33]. The establishment of the AM system might be controlled, partially, by those CSSP genes.

Trifoliate orange (*Poncirus trifoliata*) is often employed as a rootstock for citrus trees because it lacks root hairs in the field and relies heavily on AM symbiosis [34]. Meanwhile, this plant can develop well root hairs in sand culture [35]. The root morphology of sorghum plants was changed by AM fungal inoculation, resulting in a decrease in root hair density and length while increasing total root length [36]. Is there any relationship between the development of root hairs and the establishment of AM symbiosis? Although studies have been conducted to assess the role of AM fungi and root hairs in P utilization, a direct comparison of the efficiency of root hairs and AM fungi in different P levels is still required. This study investigated the interaction between P supply and AM fungal colonization on root hair development to realize: (1) the influences of P levels and AM fungal colonization on root hair morphology; (2) the effects of P levels on AM symbiosis development; and (3) the influences on the gene expression related to the root hair growth and AM symbiotic signaling.

2. Materials and Methods

2.1. Experimental Design

This experiment was designed using a randomized block design, which included two factors: (1) P supply with three levels: P-deficient (0 mg/L P, P_0), moderate P (50 mg/L P, P_{50}), and high P (500 mg/L P, P_{500}); and (2) AMF inoculation with two levels: one with *Rhizophagus intraradices* (AMF) and one with non-mycorrhizal (NM) inoculum. Each treatment was repeated 4 times, resulting in 24 pots.

2.2. Plant Materials and Growth Conditions

Nine trifoliate orange seedlings of identical size were planted in a 15 × 20 cm pot prefilled with HCl-eluted and autoclaved sand (<2 mm size) and placed in a glasshouse at 26 °C/18 °C (day/night temperature). The glasshouse had a 16 h day length and a minimum light intensity of 200 µmol quanta/m^2/s. The plants were given weekly doses of phosphate-deficient (Hoagland's solution with 0 mg P/L, P_0), moderate phosphate (half-strength Hoagland's solution with 50 mg P/L, P_{50}), and high-phosphate (Hoagland's solution with 500 mg P/L, P_{500}) solutions, respectively (pH 6.0). Half of the pots (12 pots) were inoculated with 1500 spores (80 g of inoculums consisting of spores, infected root regments, and substrates) of *R. intraradices* per pot as AMF treatment, while the other half was inoculated with the same amount of autoclaved (121 °C, 0.11 MPa, 2 h) inocula for the NM treatment. The inoculum was applied to the rhizosphere of potted seedlings. The

R. intraradices inoculums were supplied by the Bank of Glomeromycota in China. The plants were harvested and weighed at 120 days following AM fungal inoculation.

2.3. Determinations of Root AM Fungal Colonization

Six first and second lateral roots were selected from whole root systems. Root pieces having 1 cm long were pretreated using the method described by Phillips and Hayman [37]. The structures of AM fungi were examined with a compound light microscope (Olympus-BH-2, Olympos Optical Co. Ltd., Tokyo, Japan). The percentage of root length colonized by AM fungi and arbuscules was calculated following the method described by McGonigle et al. [38], with a minimal amount of 200 intersections per root sample. The quantification was carried out using the following formula:

Total AM fungal colonization rate = root length infected/root length observed × 100%;

Arbuscule colonization rate = arbuscules presence root length/root length observed × 100%;

AM fungal colonization frequency = root number infected/total root number observed × 100%.

AM dependency was expressed as the percent of AM plant dry mass against NM plant dry mass [39].

2.4. Root AM Fluorescence Staining

Root segments were treated as reported by Javot et al. [40] and stained with propidium iodide (10 μg/mL) to observe the root cell walls [41]. An argon laser was used to excite the WGA fluorescence at 488 nm, and the emission was captured using a 510 to 540 nm window. The principle investigator (PI) was excited by the 561 nm band of a diode-pumped solid-state laser, and the emission was collected through a 610 to 710 nm window. The images were captured and analyzed with a LSM510 META confocal microscope (Carl Zeiss, Jena, Germany) and accompanying software.

2.5. Root Hair Measurements

To quantify the growth of root hairs, fresh primary roots and selected first and second lateral roots were examined with an SEM (JSM-6390LV, JEOL Co., Tokyo, Japan) at magnifications of 400× and 100×, respectively. For the SEM examination, the samples were pretreated following the method described by Cao et al. [35]. The images at 5 mm intervals were recorded and processed with Image J2 software. To calculate the density of root hairs, 9 photographs were taken at 100× magnification and the surface areas of the roots were assessed with Image J. Measurements were taken on nine photographs, with ten root hairs selected at random from each image for the measurement of root hair length.

2.6. Determinations of Total P Concentration and Quantitative RT-PCR Analysis

The plant's total P concentration was measured following the method described by Li et al. [42]. Gene expression profiles were determined using the Roche Light Cycler 480 System, according to the referencing method described by Wang et al. [43]. The primers (Supplementary Material Table S1) for qRT-PCR were designed using Primer Premier 5 software and synthesized by Sangon Biological Engineering Technology & Service Co., Ltd. (Shanghai, China). β-Actin was used as a housekeeping gene.

2.7. Data and Statistical Analysis

The experimental data underwent a two-way ANOVA analysis with AM fungi and P supply as factors. The percentage data were transformed using arcsin x 1/2. To evaluate treatment significance ($p < 0.05$), probabilities of significant difference were used, followed by LSD multiple comparison tests with SAS 9.1 software.

3. Results

3.1. Changes in Root Hair Density and AM Fungal Colonization

Under P deficit, the density and length of root hairs in NM plants increased significantly (Table 1). Additionally, plants under high P conditions exhibited fewer but longer root hairs than moderate P-treated plants, with *R. irregularis* increasing both density and length. The length of epidermal cells in P-deficient NM plants underwent a significant decrease, but it was increased by AMF colonization, while the epidermal cell number was just the opposite. Under high P conditions, the elongation of epidermal cells was induced, but the epidermal cell number was reduced markedly, along with no effect on epidermal cell length.

Table 1. Root hair density and length, epidermal cell length, and number of AMF or NM trifoliate orange seedlings grown under P-deficient (P_0), moderate P (P_{50}), and high P (P_{500}) conditions.

Treatments		Root Hair Density (num./mm^2)	Root Hair Length (μm)	Epidermal Cell Length (μm)	Epidermal Cell Number
NM	P_0	131.50 ± 4.16 a	33.30 ± 0.47 a	10.09 ± 0.22 d	110 ± 8 a
	P_{50}	100.61 ± 0.52 c	29.03 ± 0.93 c	11.18 ± 0.37 c	103 ± 1 b
	P_{500}	58.70 ± 4.74 e	31.63 ± 0.12 b	12.13 ± 0.29 b	100 ± 6 bc
AMF	P_0	111.42 ± 1.18 b	30.97 ± 0.99 b	12.95 ± 0.33 a	95 ± 1 c
	P_{50}	113.18 ± 2.28 b	28.80 ± 0.44 c	11.96 ± 0.63 bc	102 ± 6 b
	P_{500}	85.06 ± 3.958 d	34.278 ± 0.45 a	12.31 ± 0.76 ab	100 ± 5 bc
ANOVA					
P levels		***	***	*	NS
AMF		**	NS	***	**
Interaction		***	***	***	**

Means of six observations followed by the same letter within a column are not significantly different among treatments at $p < 0.05$. Data were analyzed with ANOVA. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$. NS, not significant at $p < 0.05$.

Roots of trifoliate orange achieved a higher level of AM fungal colonization of 75.8 ± 2.3% at P deficit but only 53.2 ± 1.0% by AMF under high P levels (Figure 1). Similarly, arbuscules were barely (5.8 ± 0.1%) observed under high P conditions, but had a higher level (12.9 ± 0.4%) at P deficit. The colonization frequency (percent of infected roots against total observed roots), was also significantly greater at P deficit but lower at high P levels.

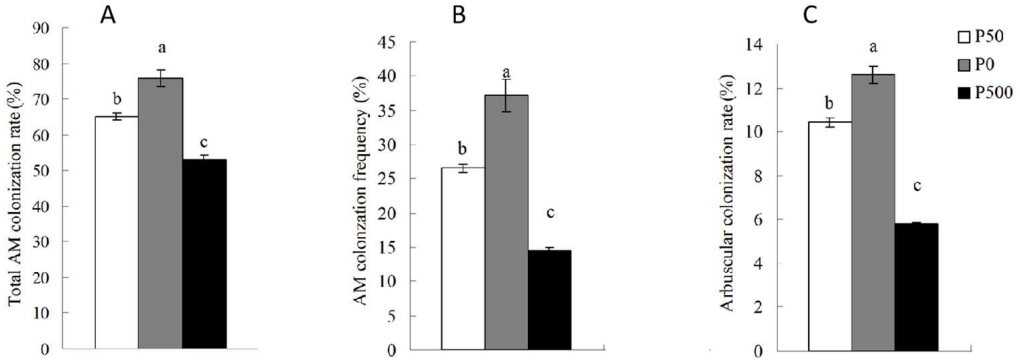

Figure 1. Total AMF colonization rate (**A**), AMF colonization frequency (**B**), and arbuscule colonization rate (**C**) in the lateral roots of AMF or NM trifoliate orange seedlings grown under P-deficient (P_0), moderate P (P_{50}) and high P (P_{500}) conditions. The data are means ± SD of six independent replications run in quadruplicate; for the analysis of variance, different small letters indicate statistically significant differences between treatments in the first or second lateral root ($p < 0.05$).

SEM and LSCM were used to observe the root hair and arbuscular mycorrhizal morphology (Figure 2). In addition, the roots of trifoliate orange were investigated with confocal and electron microscopy. The different stages of mycorrhizal structure formation are presented in Figure 3: (1) the hyphae contacted plant root surface and formed appressorium; (2) the hyphae colonized and formed tree branches within the cell; (3) the hyphae grew rapidly and extended along the column; (4) a vesicle formed; (5) the spore began to form and coexist with the hyphae; and (6) the spores largely formed, and the hyphae gradually tapered.

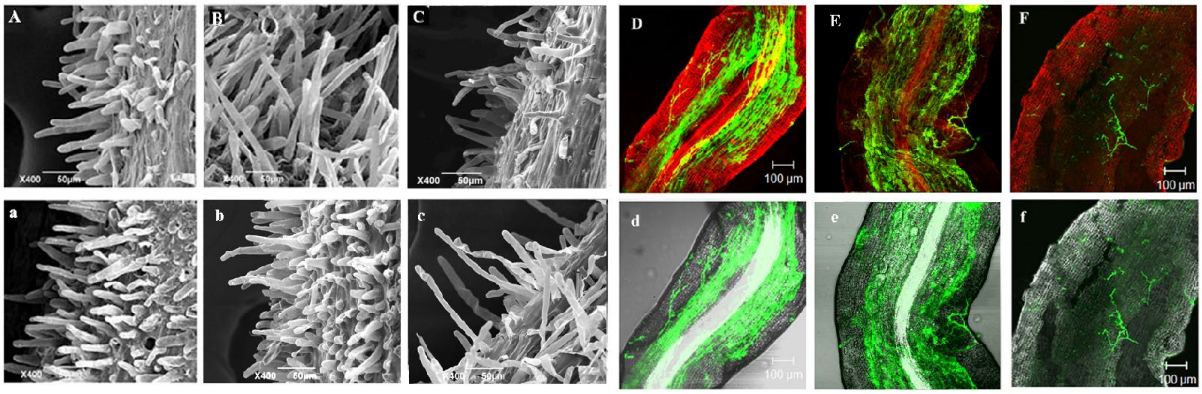

Figure 2. The root hair and arbuscular mycorrhizal morphology of trifoliate orange seedlings fertilized with moderate P (P_{50}: (**A**,**a**,**D**,**d**)), P-deficient (P_0: (**B**,**b**,**E**,**e**)), and high P (P_{500}: (**C**,**c**,**F**,**f**)) levels. (**A**–**C**): Scanning electron microscope (SEM) images of root hairs on NM plants; (**a**–**c**): SEM images of root hairs on AMF plants. Bar = 50 μm. Laser scanning confocal microscope images of arbuscular mycorrhizal: bright and green fluorescence overlay micrographs showing staining of fungal structure with Alexa Fluor 488 WGA (**D**–**F**); red and green fluorescence overlay micrographs showing staining of fungal structure with Alexa Fluor 488 WGA (**d**–**f**). Bar = 100 μm.

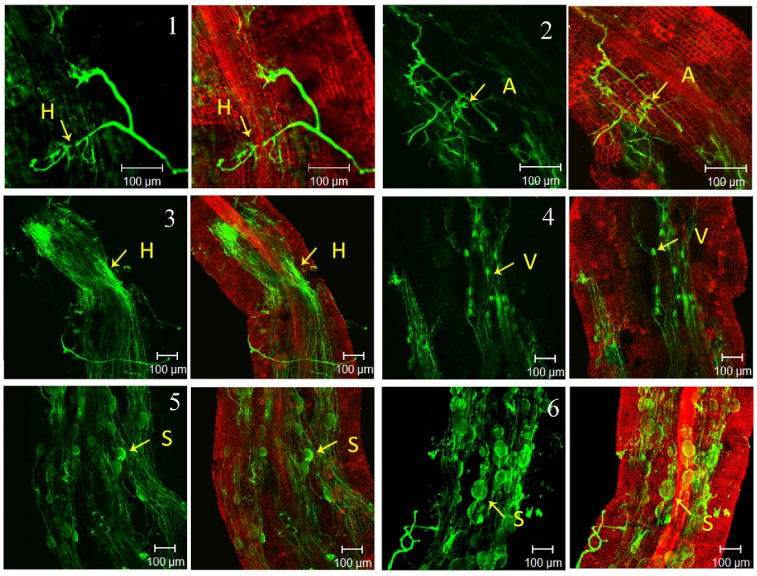

Figure 3. Different stages of arbuscular mycorrhizal in trifoliate orange lateral roots under P-deficient conditions. On the left, there are green fluorescence micrographs showing the staining of fungal structure with Alexa Fluor 488 WGA, and on the right, there are red and green fluorescence overlay micrographs showing the staining of fungal structure with Alexa Fluor 488 WGA in each stage. H = hyphae; A = arbuscules; V = vesicle; S = spores.

3.2. Effects of AM Fungi and P Supply on P Content and Plant Growth

Non-AM plants accumulated 2.0 and 2.4 times more P under high P conditions than they did under moderate P and P-deficient situations (Table 2). P accumulation in AMF plants increased by 50.7% and 18.1%, respectively, under P-deficient and moderate P conditions, while there was no notable difference between AMF and NM seedlings at high P levels. Significant differences in biomass were observed between plants treated with different levels of P, regardless of whether they were NM or AMF plants (Table 2). The NM plants under P-deficient conditions had smaller shoot and root dry mass compared with the plants grown under moderate P and high P conditions, with the total dry mass being 11% and 26%, respectively. However, AMF inoculation increased dry mass greatly under P-deficient conditions, but only somewhat under the other two P supply situations. Therefore, the mycorrhizal dependency presented the following trend: P-deficient condition > moderate P condition > high P condition.

Table 2. Plant total P content, dry mass and mycorrhizal dependency of AMF or NM trifoliate orange seedlings grown under P-deficient (P_0), moderate P (P_{50}) and high P (P_{500}) conditions.

Treatments		Total P Content (mg/g)	Shoot Dry Mass (g)	Root Dry Mass (g)	Totle Dry Mass (g)	Mycorrhizal Dependency (%)
NM	P_0	0.69 ± 0.05 d	0.67 ± 0.04 e	0.14 ± 0.01 e	0.81 ± 0.05 e	
	P_{50}	0.83 ± 0.05 c	0.75 ± 0.05 d	0.19 ± 0.04 d	0.94 ± 0.02 d	
	P_{500}	1.64 ± 0.04 a	0.91 ± 0.02 c	0.22 ± 0.02 cd	1.14 ± 0.04 c	
AMF	P_0	1.04 ± 0.06 b	1.48 ± 0.02 a	0.48 ± 0.02 a	1.96 ± 0.03 a	241.98 ± 8.17 a
	P_{50}	0.98 ± 0.03 b	1.26 ± 0.05 b	0.36 ± 0.01 b	1.62 ± 0.05 b	172.34 ± 5.85 b
	P_{500}	1.68 ± 0.08 a	0.92 ± 0.03 c	0.24 ± 0.03 c	1.16 ± 0.05 c	101.70 ± 1.28 c
ANOVA						
P levels		***	***	***	***	***
AMF		***	***	***	***	-
Interaction		***	***	***	***	-

Means of six observations followed by the same letter within a column are not significantly different among treatments at $p < 0.05$. Data were analyzed with ANOVA. ***, $p < 0.001$.

3.3. Effects of AM Fungi and P Supply on the Expression of Genes Associated with Root Hair Formation

To investigate the impact of P supply conditions and AMF colonization on the genes responsible for root hair formation, we analyzed the expression levels of genes associated with root hair development and cell fate. In NM plants, genes like *ROP1*, *ROP2*, *ROP9*, and *LRX1*, crucial for the development of root hair, were 1.4- to 3.1-fold higher expressed under P_0 and P_{500} than under P_{50}, and the cell fate-determined genes, such as *TRY*, *WER*, *CPC*, and *TTG*, were also strongly expressed, with the *WER* gene reaching 45.4–56.7-fold (Figure 4). The expression levels of the *FER* gene were strongly upregulated at P_0 and P_{500} in NM plants, but this upregulation was reversed when AMF was inoculated into the plants. The results indicated that root hair development and cell fate-related genes were induced to express under both P-deficient and high P conditions. In AMF plants, higher expression of *ROP1*, *ROP2*, *ROP9*, *LRX1*, *TRY*, *CPC*, and *GL2* genes intensified at P_{500} compared with that in NM plants, whereas the *WER* and *TTG* genes were reduced. The *ROP1* gene expression in AMF-treated plants showed a significant increase at P_0 compared with non-mycorrhizal plants, whereas the relative expression of *ROP2*, *ROP9*, *LRX1*, *TRY*, *CPC*, *WER* and *TTG* genes decreased (Figure 4). The findings indicated that the impact of AMF colonization on the regulation of genes related to root hair formation was dependent on P availability, with AMF colonization, increasing the expression of most of these genes at high P levels but inhibiting it at low P levels.

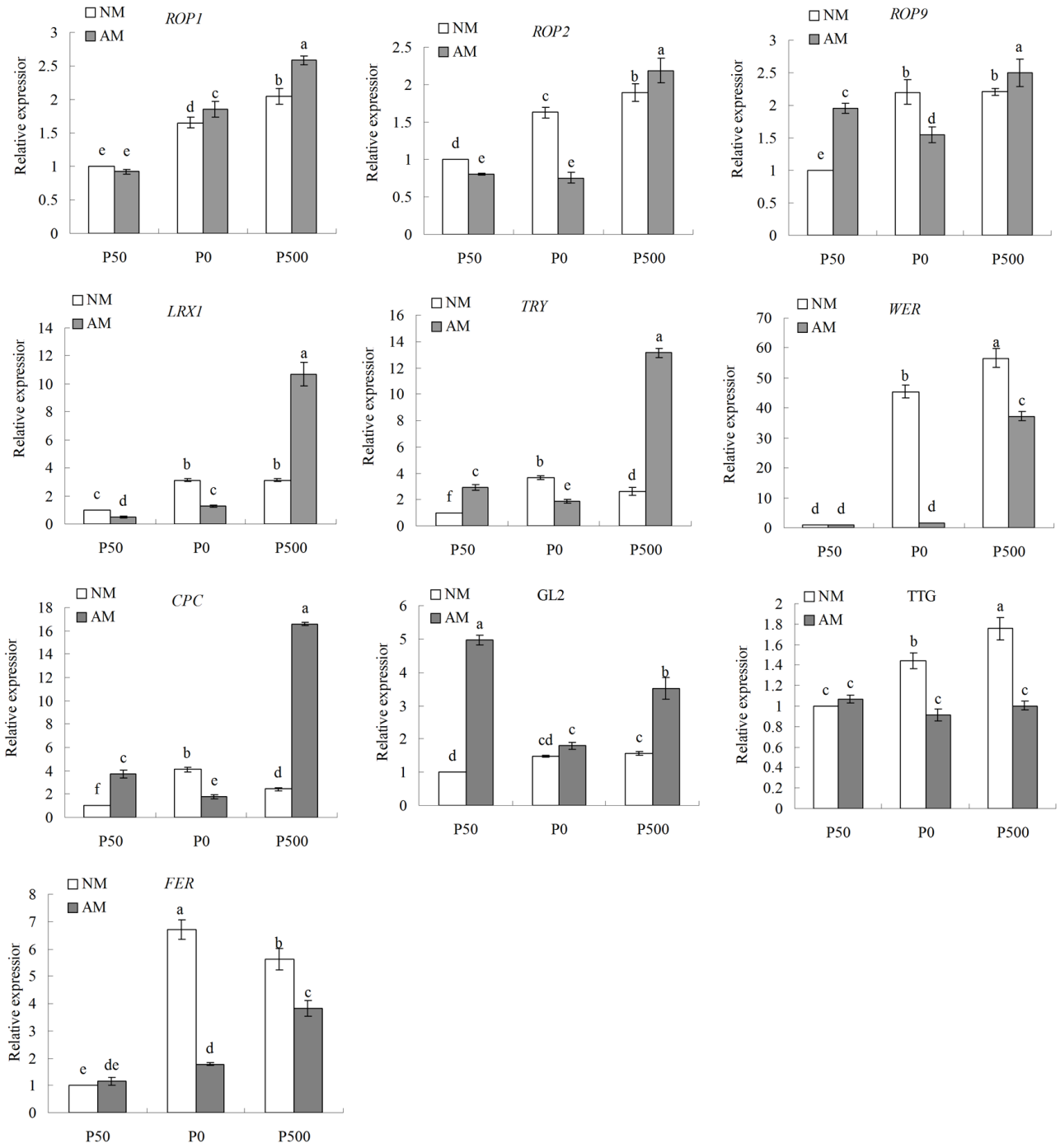

Figure 4. Variation in the expression of root hair development-related genes of trifoliate orange seedlings fertilized with moderate P (P_{50}), P-deficient (P_0), and high P (P_{500}) solutions. Plants were grown in microcosms inoculated with arbuscular mycorrhizal fungi (AMF) or with non-mycorrhizal (NM) inoculum. The data are the means ± SD of six independent replications run in quadruplicate. Different letters in bars indicate significant ($p < 0.05$) differences between treatments by LSD tests.

3.4. Effects of AM Fungi and P Supply on the Expression of Genes Associated with AM Signaling

The AM signal gene (including *CASTOR*, *DMI1*, *DMI2*, *DMI3*, *NUP85*, *NUP133*, and *CYCLOPS*) expression levels were boosted at P_0 and were found to be 1.2–1.9-fold higher expressed than at P_{50}, whereas the expression of *DMI3*, *NUP85*, *NUP133*, and *CYCLOPS* was inhibited at P_{500} (Figure 5). This study suggested that P-deficient conditions increased

the expression of AM signaling genes, while high P conditions suppressed the expression of genes downstream from this pathway.

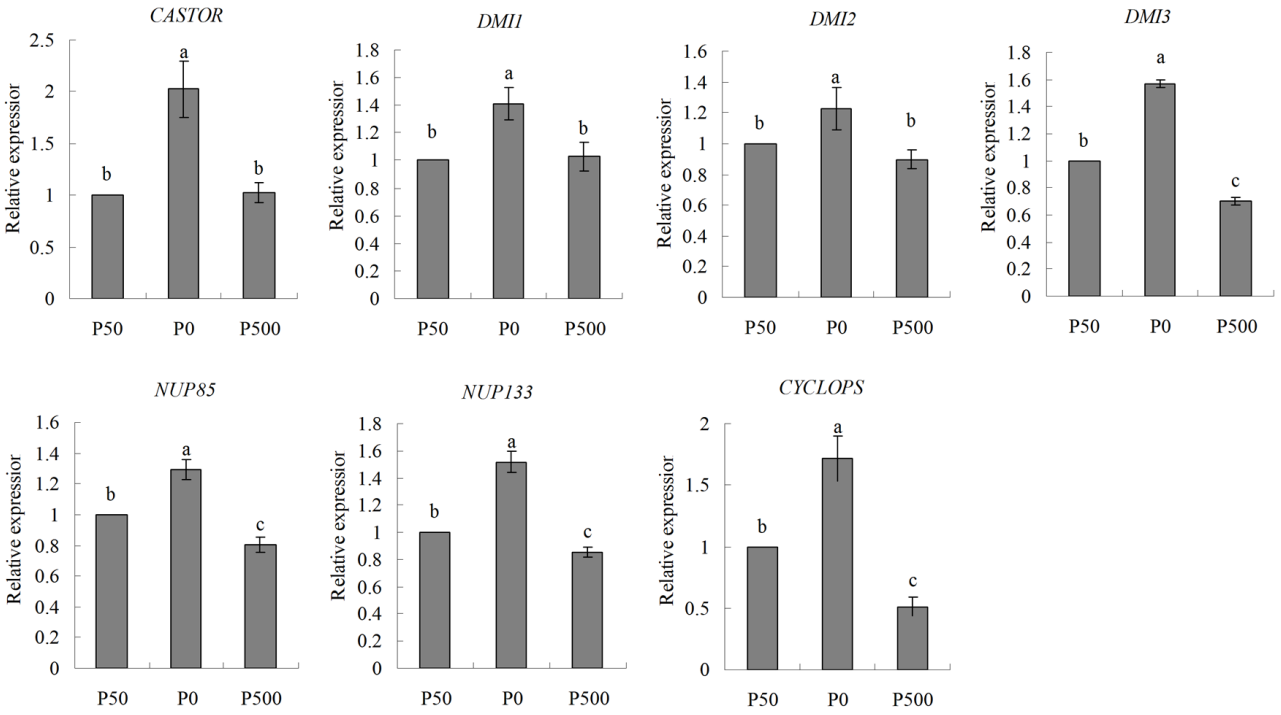

Figure 5. Variation in the expression of AM signaling genes of trifoliate orange roots fertilized with moderate P (P_{50}), P-deficient (P_0), and high P (P_{500}) solutions. The plants were grown in microcosms inoculated with arbuscular mycorrhizal fungi (AMF) or with non-mycorrhizal (NM) inoculum. The data are the means ± SD of six independent replications run in quadruplicate. Different letters in bars indicate significant ($p < 0.05$) differences between treatments by LSD tests.

4. Discussion

4.1. AM Fungal Inoculation and P Supply Conditions Influence Root Hair Morphology and Expression of Root Hair Development Genes

In this experiment, the root hair density and length rose significantly under P-deficient conditions while decreasing under high P conditions. This finding is consistent with previous studies on *Arabidopsis thaliana*, corn, and wheat [44,45]. Furthermore, the elongation of root epidermal cells in trifoliate orange was inhibited under P-deficient conditions, although the number of epidermal cells increased. It is well known that root hairs are elongated structures that protrude from epidermal cells, and a deficiency in P can enhance root hair growth by increasing the quantity of epidermal cells [46]. Plants can regulate the rhizosphere soil microorganisms, while fungal exudates regulate seed germination and root development [47]. Both *Gigaspora rosea* and *Frankia* could increase root branching in *Alnus glutinosa* but decrease root hair frequency markedly [48]. Similarly, we observed that root hair length and frequency were promoted by AMF colonization at high P levels but suppressed at P-deficient levels, suggesting that mycorrhizal influence on root hair development depended on substrate P levels. Meanwhile, the epidermal cells were reduced by AMF colonization under P-deficient conditions, indicating that AMF colonization suppressed root hair development by reducing the number of epidermal cells. It has been demonstrated that root hairs are more efficient in increasing P acquisition under P deficiency conditions as root hair length and AM fungal colonization are negatively related and root hair development takes absolute advantage [49]. However, the symbiosis between AM fungi and trifoliate orange was found to be stronger in P-deficient versus moderate and high P levels. This result is similar to a study by Wu et al. [50] that investigated the symbiosis between trifoliate orange and *Funneliformis mosseae* under varying P levels. Therefore,

increased AM development of trifoliate orange in P deficit may limit the development of root hairs, indicating that resources are allocated to AM symbiosis rather than root hairs in response to P deficiency. However, root hairs were stimulated by AMF inoculation under high P levels. The results clearly support the idea that plants may represent alternative strategies to increase P acquisition under various P situations.

Studies have demonstrated that a wide range of genes are involved in root hair remodeling and are activated by various P conditions. Specifically, 1118 protein kinase (PK) and 205 phosphatase (PP) genes were found to be expressed differently in response to P deficiency [51]. FER is essential for root hair initiation and elongation, whether in the default developmental pathway or induced by exogenous auxins. The *OsPHR2* gene, which is implicated in the P starvation response, controls the growth of root hairs and root elongation. Overexpression of *OsPHR2* mitigated Pi-starvation stress by boosting root elongation and the number of root hairs [52]. In this study, the expression of root hair cell fate, tip growth and initiation genes were influenced by AMF colonization, P-deficient, or high P levels. Under high P conditions, induced expression of atrichoblast-specific genes (*WER* and *TTG*) may increase the non-root hair cells, ultimately leading to a considerable drop in root hair density and length. Meanwhile, the presence of *R. intraradices* may promote root hair cell destiny by increasing expression of trichoblast-specific genes (*TRY* and *CPC*) and atrichoblast-specific genes (*WER* and *TTG*). Moreover, under P-deficient conditions, AMF colonization suppressed root hair development. It is due to the limited expression of genes (*ROP2*, *ROP9*, *LRX1*, and *FER*) related to root hair initiation and elongation in AM plants. The results are in concordance with other reports showing that the development of root hair, partially, was controlled by those cell-type-specific genes [6,53,54].

4.2. P Supply Levels Affect AM Symbiosis Establishment and the Expression of AM Signaling Genes

Interestingly, AMF colonization exhibited a similar reaction with root hairs to P supply conditions as the AMF colonization rate, and the intercellular structure under P deficiency was superior to that under high P conditions. It is assumed that root hairs play a significant role in the initial stages of mycorrhizal development because they are sensitive to chemicals generated by AMF and serve as a favored colonization site. The root hairs serve as a preferential attachment site [55]. Therefore, the modification of root hair development may be an effective way for P supply to regulate AM formation in trifoliate orange. Furthermore, P supply could also directly affect AMF colonization by regulating the expression of AM signaling genes. In this study, seven genes (e.g., *CASTOR*, *DMI*, *NUP*, and *CYCLOPS*) in the AM signaling pathway were upregulated at P-deficient levels, whereas the genes downstream from this pathway (e.g., *DMI3*, *NUP85*, *NUP133*, and *CYCLOPS*) were downregulated at high P levels.

4.3. Interactions between Root Hair Development, AM Symbiosis Establishment, and Plant P Absorption

Root hairs are responsible for acquiring mineral nutrients and anchoring the plant in the soil [56]. Root hairs are not the sole structures responsible for nutrient acquisition; mycorrhizal fungi also play a role in helping their host plants absorb nutrients [34,57,58]. In this study, we created a network among the nutrient supply conditions (P), AMF colonization, and plants (root hairs). There are two distinct response models under P-deficient and high P conditions. First, under high P conditions, the atrichoblast cell (non-root hair cell) increased due to upregulating expression of atrichoblast-specific genes (*WER* and *TTG*), suppressing root hair development and further suppressing AM symbiosis development. In contrast, the downregulated AM signaling genes (*NUP85*, *NUP133*, and *CYCLOPS*) directly suppressed the establishment of AM symbiosis under high P conditions. Meanwhile, AMF infection initiated root hair development by increasing trichoblast cells (root hair cells) due to upregulating expression of trichoblast-specific genes and downregulating expression of atrichoblast-specific genes. In the second model, epidermis cell number increased due to impaired cell elongation, leading to increased root hair density and indirect AMF colo-

nization under P-deficient conditions. In addition, AMF colonization was improved under P-deficient conditions, owing to increased expression of AM signaling genes. Moreover, AMF colonization not only stimulated the elongation of epidermis cell and reduced the epidermis cell number but also declined the expression of root hair tip growth and initiation genes, leading to inhibition of root hair development (Figure 6).

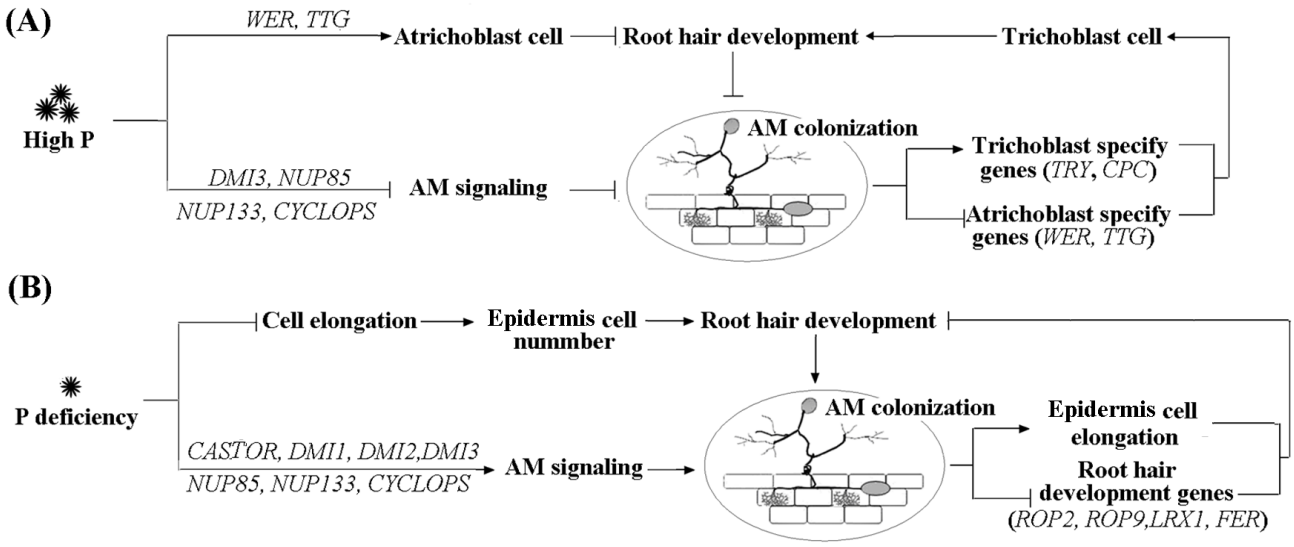

Figure 6. Schematic representation of the interaction between root hair development and AM system establishment under high P (**A**) and P deficiency conditions (**B**). *TRY* and *CPC* are trichoblast-specific genes; *WER* and *TTG* are atrichoblast-specific genes; *ROP2*, *ROP9*, *LRX1*, and FER genes are involved in root hair initiation and (or) tip growth; *CASOTOR*, *DMI1*, *DMI2*, *DMI3*, *NUP85*, *NUP133* and *CYCLOPS* are involved in AM signaling; the symbols "T" and "↓" represent the effects of inhibition and activation, respectively.

5. Conclusions

The present study proposed a network for the link between AM symbiosis establishment and root hair development at different P levels: (1) substrate P levels and AMF inoculation could remodel root hair morphology; (2) the development of root hair (the preferential colonization site) had a major influence on the AM formation; (3) AMF colonization promoted the elongation of epidermal cells while inhibiting the growth of root hair tips, resulting in fewer epidermal cells and lower expression of genes involved in root hair initiation. This ultimately slowed the development of root hairs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14050763/s1, Table S1: Oligonucleotide primer of selected genes for qRT-PCR.

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