

# 丛枝菌根通过调节碳磷代谢相关基因的表达增强植物对低磷胁迫的适应性

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**摘 要** 丛枝菌根(AM)共生体系对于植物适应低磷胁迫具有重要作用。AM不仅直接调节宿主植物对低磷胁迫的响应, 还可能通过分泌物影响相邻的非菌根植物。该研究采用分室培养系统, 以玉米(*Zea mays*)和AM真菌*Rhizophagus irregularis*为试验材料, 考察低磷(10 mg·kg<sup>-1</sup>)和高磷(100 mg·kg<sup>-1</sup>)条件下, 菌根共生体系对植物生长、磷营养以及碳磷代谢相关基因表达的影响, 以揭示AM调节植物低磷胁迫响应的生理机制。分室培养系统由0.45 μm微孔滤膜分隔成供体室、缓冲室和受体室3个分室, 以供体室菌根化植物为AM分泌物来源, 通过微孔膜阻止菌根真菌对未接种受体植物的直接影响, 但允许AM分泌物在分室间的扩散。采用实时荧光定量PCR技术分析玉米以及AM真菌自身碳磷代谢相关基因的表达情况。试验结果表明, 低磷条件下接种AM真菌显著提高了供体植物干质量和磷浓度, 上调了玉米碳磷代谢相关基因的表达。AM真菌磷转运蛋白基因和碳代谢相关基因在低磷条件下的表达水平显著高于高磷水平; 对于受体植物而言, 仅高磷处理显著提高了玉米植株干质量和磷含量, 而接种处理显著上调了受体植物磷转运蛋白基因和碳代谢相关基因的表达水平。该研究表明, 低磷胁迫下AM可能通过分泌物调控植物碳磷代谢相关基因的表达, 进而调节植物对低磷胁迫的生理响应。

**关键词** 分室培养系统; 丛枝菌根; 碳磷代谢; 功能基因; 缺磷胁迫

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## Arbuscular mycorrhiza improves plant adaptation to phosphorus deficiency through regulating the expression of genes relevant to carbon and phosphorus metabolism

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### Abstract

**Aims** Arbuscular mycorrhizal (AM) symbiosis plays an important role in plant adaptation to phosphorus (P) deficiency. The mycorrhizal fungi can directly regulate P stress response of the host plants, and can also indirectly influence neighbor plants via AM exudates. This study aimed to reveal the regulation mechanisms of plant response to P deficiency by AM associations.

**Methods** In a compartmentation cultivation experiment with *Zea mays* ‘B73’ and AM fungus *Rhizophagus irregularis* ‘DAOM197198’, we investigated mycorrhizal effects on plant P nutrition and the expression of plant and fungal genes related to P and carbon (C) metabolisms under both low P (10 mg·kg<sup>-1</sup>) and high P (100 mg·kg<sup>-1</sup>) conditions. The cultivation system consisted of three compartments, namely donor compartment, buffer compartment and receiver compartment divided by two pieces of microporous filters with pore size of 0.45 μm. Maize plant in donor compartment inoculated with AM fungus served as a source of AM exudates. The microporous filters could restrict the development of extraradical mycelium of AM fungi, but allow diffusion of AM exudates. Real-time PCR was performed to quantify the gene expression levels both in maize plants and AM fungi.

**Important findings** The experimental results indicated that under low P conditions mycorrhizal colonization increased plant dry weight and P concentration in donor plants, and up-regulated plant genes encoding P

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transporters *Pht1;2*, *Pht1;6*, phosphoenolpyruvate carboxylase (*PEPC*), inorganic pyrophosphatase (*TC289*), glycerol-3-phosphate transporter (*G3PT*) and malate synthase (*MASI*). The expression of AM fungal genes encoding P transporter (*GiPT*), GlcNAc transporter (*NGT1*), GlcNAc kinase (*HXK1b*), GlcNAc phosphomutase (*AGM1*), UDP GlcNAc pyrophosphorylase (*UAP1*), chitin synthase (*CHS1*), GlcNAc-6-phosphate deacetylase (*DAC1*) and glucosamine-6-phosphate isomerase (*NAG1*) was significantly higher under low P conditions compared with high P conditions. However, for the receiver plants, plant dry mass and P concentration were only significantly increased by higher P addition, while inoculation treatment significantly up-regulated the expression of P transporter genes *Pht1;2* and *Pht1;6*, C metabolism related genes *G3PT*, *PEPC*, *TC289* and *MASI*. The study proved that AM exudates could potentially stimulate plant response to P deficiency by regulating functional genes relevant to P and C metabolisms in the mycorrhizal associations.

**Key words** compartment cultivation system; arbuscular mycorrhiza; carbon and phosphorus metabolism; functional gene; phosphorus deficiency

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在热带和亚热带酸性土壤及温带钙化土壤中, 土壤有效磷含量很低, 而且施用的磷肥很快会被土壤固定, 难以被植物吸收利用(Fixen, 2002)。在作物生长发育的早期, 磷元素的获取至关重要(Plénet *et al.*, 2000), 因此低磷胁迫成为制约作物生产的重要因素。在漫长的进化过程中, 植物也进化出一系列分子和生化机制来适应低磷胁迫。有研究表明, 玉米(*Zea mays*)根系中的多个功能基因, 如与糖酵解相关的甘油-3-磷酸转运蛋白基因(*G3PT*)、磷酸烯醇式丙酮酸羧化酶基因(*PEPC*)、三羧酸循环相关基因苹果酸合酶基因(*MASI*)和无机焦磷酸化酶基因(*TC289*)等的表达水平在低磷胁迫下明显增高, 在改善植物磷营养方面具有重要作用(Carlos *et al.*, 2008)。此外, 大多数植物可以与土壤中的丛枝菌根(AM)真菌形成共生体系。AM真菌帮助宿主植物吸收土壤中的磷, 而植物提供5%–20%的光合产物帮助AM真菌完成生活史(Wright *et al.*, 1998)。研究表明, AM共生体系对植物适应低磷胁迫具有重要的积极作用。在植物中存在菌根特异性和菌根诱导性两种磷转运蛋白, 低磷环境下菌根上调磷转运蛋白的表达水平, 促进植物高效吸收利用土壤中的磷(Harrison *et al.*, 2002; Casieri *et al.*, 2013)。不同作物中的磷转运蛋白基因, 如玉米*Pht1;6* (Nagy *et al.*, 2009)、土豆(*Solanum tuberosum*) *Pht1;3*、番茄(*Solanum lycopersicum*) *Pht1;4* (Nagy *et al.*, 2005)和水稻(*Oryza sativa*) *Pht1;13* (Guimil *et al.*, 2005)在AM真菌侵染时会被强烈诱导表达上调。另一方面, 在AM真菌中已有3种磷转运蛋白(*GvPT*、*GiPT*和*GmosPT*)被鉴定出来。在缺磷的情况下, 这些磷转

运蛋白会在根外菌丝中强烈表达, 促进真菌吸收磷并传输给宿主植物(Maldonado-Mendoza *et al.*, 2001)。

宿主植物和AM真菌之间的碳磷交换是稳定菌根共生体系的基础(Bago *et al.*, 2000)。目前, 关于菌根促进植物吸收磷的机理研究已有很多, 但是菌根真菌自身碳代谢研究还比较少。N-乙酰葡萄糖胺(GlcNAc)代谢是AM真菌中一种比较重要的碳代谢过程, 不仅参与几丁质的合成, 还为AM真菌的糖酵解过程提供前体物质, 真菌细胞壁的特征性组分几丁质即是GlcNAc的长链聚合物(Rich *et al.*, 2014)。最近, Yoshihiro等(2015)构建了AM真菌中GlcNAc代谢通路: AM真菌菌丝中的GlcNAc转运蛋白NGT1能够将外源GlcNAc转运到菌丝内部, 继而后由GlcNAc激酶a和激酶b (HXK1a和HXK1b)将GlcNAc磷酸化为GlcNAc-6-磷酸, 之后, GlcNAc-6-磷酸有两个下游通路, 一个是GlcNAc磷酸变位酶AGM1、UDP-GlcNAc焦磷酸化酶UAP1和几丁质合酶CHS1参与的几丁质合成代谢; 另一个是GlcNAc-6-磷酸去乙酰化酶DAC1和葡萄糖胺-6-磷酸异构酶NAG1作用形成果糖-6-磷酸进入糖酵解途径的分解代谢。考察磷胁迫下AM真菌中这些碳代谢相关基因表达的变化, 有助于更好地揭示菌根共生体系适应低磷胁迫的生理机制。

AM不仅能够直接参与和调节宿主植物碳磷代谢过程, 还可能通过菌根根际效应(mycorrhizosphere effects)调节相邻非菌根植物的生理代谢和生长发育(van der Heijden & Horton, 2009)。Barto等(2011)利用H形盆栽装置构建了可调控的菌根网络(common mycorrhizal networks, CMNs), 验证了CMNs在不同

植株之间传输化感物质的可能性。然而, 在根外菌丝完全不接触未接种植物的情况下, AM是否能够通过菌根分泌物作用调节植物的生理代谢还未见研究报道。基于此, 本试验采用分室培养系统, 在不同供磷条件下考察AM对宿主植物和相邻非菌根植物生长和生理的影响。在由供体室、缓冲室和受体室组成的三分室培养系统中, 采用微孔滤膜区隔不同分室, 可阻断接种处理供体植物根外菌丝与受体植物的直接联系, 从而考察来源于供体植物的AM分泌物对受体植物的可能作用。通过观测供体和受体植物生物量、磷浓度, 分析植物及AM真菌碳磷代谢相关基因的表达, 探讨AM直接和间接调控植物响应低磷胁迫的生理机制。

## 1 材料和方法

### 1.1 试验材料

供试植物为已完成全基因组测序的模式作物材料玉米自交系‘B73’ (Schnable *et al.*, 2009)。玉米种子由中国农业大学生物学院于静娟教授提供。供试AM真菌为根内球囊霉 *Rhizophagus irregularis* ‘DAOM197198’, 接种剂为取自转移Ri T-DNA胡萝卜 (*Daucus carota* var. *sativa*) 根器官和AM真菌双重无菌培养体系的真菌孢子和被侵染的胡萝卜根段。

供试土壤采自内蒙古鄂尔多斯市东胜区铜川镇积机塔村(39.89° E, 110.02° N, 海拔1 367 m)。土壤基本理化性质如下: pH值8.69 (水浸提, 水土质量比2.5:1), 有机质含量22.01 g·kg<sup>-1</sup>; 有机碳含量12.77 g·kg<sup>-1</sup>; 有效磷含量4.46 mg·kg<sup>-1</sup>, 具体测定方法参见鲍士旦(2000)。土壤过2 mm筛后, 辐照灭菌

(<sup>60</sup>Co, 25 kGy)。培养基质由灭菌土壤与河沙按质量比1.5:1混合组成。试验前土壤加入底肥NH<sub>4</sub>NO<sub>3</sub>-N 90 mg·kg<sup>-1</sup>, K<sub>2</sub>SO<sub>4</sub>-K 120 mg·kg<sup>-1</sup>。低磷处理加NaH<sub>2</sub>PO<sub>4</sub>-P 10 mg·kg<sup>-1</sup>, 高磷处理加NaH<sub>2</sub>PO<sub>4</sub>-P 100 mg·kg<sup>-1</sup>。分室培养系统采用2 mm厚的硬聚氯乙烯(PVC)板加工制成, 由2层0.45 μm微孔滤膜分成3个分室(AM分泌物供体室、缓冲室和受体室), 供体室和受体室体积相同并种植玉米, 中间为宽2 cm的缓冲室。培养系统尺寸(长×宽×高)为((11 + 2 + 11) × 11 × 12.5) cm<sup>3</sup> (图1)。

### 1.2 试验方案

供体室玉米接种AM真菌, 作为AM分泌物的源, 同时设置不接种对照处理。受体室不进行接种处理。不同接种处理下均设低磷(10 mg·kg<sup>-1</sup>)和高磷(100 mg·kg<sup>-1</sup>)处理(所有分室磷浓度相同)。试验共有4个处理, 每个处理重复3次, 共12盆。玉米播种59天后进行试验收获。供体室接种AM真菌情况下, 供体室标记为AMD (AM Donor), 受体室标记为AMR (AM Receiver), 不接种情况下(non-mycorrhizal, NM), 供体室和受体室分别标记为NMD和NMR (图1)。

### 1.3 植物培养与试验管理

‘B73’种子经清水浸泡3 h, 10%过氧化氢浸泡15 min后, 取出并用灭菌水冲洗数遍后放入装有3层湿润滤纸的培养皿中, 25 °C催芽4天。供体室和受体室分别装入1 500 g施加过底肥的培养基质, 缓冲室装入250 g相同的培养基质。称质量浇水使土壤含水量达到14%质量含水量, 待水分渗透均匀后, 播入萌发的玉米种子。将2 000个AM真菌孢子附着在已萌发玉米种子的胚根部位, 并加入0.08 g风干的

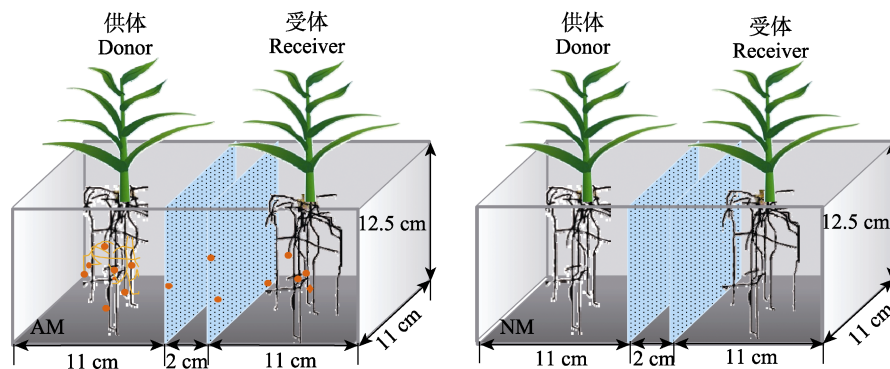


图1 分室培养系统示意图。以0.45 μm微孔滤膜区隔不同分室。AM和NM分别代表供体植物接种AM真菌和不接种对照处理。处理分为高磷和低磷(10 mg·kg<sup>-1</sup>和100 mg·kg<sup>-1</sup>)两个水平, 每个处理三个重复(n = 3)。

Fig. 1 Diagram of the compartment cultivation system. Different compartments were separated by microporous filter with pore size of 0.45 μm. AM and NM represent inoculation of donor plants with AM fungus and the non-mycorrhizal control respectively. There are two phosphorus levels (10 mg·kg<sup>-1</sup> and 100 mg·kg<sup>-1</sup>), and three replications for each treatment (n = 3).

AM侵染的胡萝卜根段; 不接种处理加入0.08 g风干的无菌根侵染的胡萝卜根段。

盆栽试验在人工气候室中完成, 温度为25 °C/20 °C, 每天光照16 h, 光强约700  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , 相对湿度为70%。在整个试验周期都维持14%的土壤含水量, 每天称质量补足损耗的水量, 使土壤含水量维持在设定水平。

## 1.4 测定指标和方法

### 1.4.1 生物量及菌根侵染率的测定

试验收获时, 自培养基表面将植物剪断。将玉米地上部和根系用去离子水冲洗两遍, 吸干表面水分, 分别称取鲜质量。取混匀的玉米根系 0.1 g, 经液氮冷冻后, 于-80 °C保存, 用于RNA提取; 另取混匀根系样品3 g, 用于菌根侵染率测定。余下样品置于105 °C烘箱中杀青10 min, 转为80 °C烘干至恒质量, 称干质量。根系染色过程依据Phillips和Hayman (1970)的方法, 但有所简化: 将3 g根系放入10% KOH中, 90 °C水浴锅中煮10 min, 用自来水冲洗干净后, 加入0.05%的台盼蓝90 °C染色5 min。染色根段置于载玻片上, 然后加盖盖玻片观察, 每个样品观测30根段(李涛和陈保冬, 2012)。用根段频率法计算菌根侵染率(Biermann & Linderman, 1981)。

### 1.4.2 植物磷浓度的测定

取烘干的植物样品, 经粉碎机粉碎后, 称取样品约0.1 g, 以5 mL优级纯 $\text{HNO}_3$ 室温消化12 h后, 在微波消解仪(MARS5, CEM, Matthews, USA)中进行消解, 消解液定容至25 mL, 混匀后过滤, 用电感耦合等离子原子发射光谱仪(ICP-OES, Leeman Labs, Hudson, USA)测定样品中的磷浓度。

### 1.4.3 玉米和AM真菌碳磷代谢相关基因表达测定

取0.1 g玉米根部样品, 采用TRIZOL试剂(Invitrogen, Grand Island, New York, USA)提取玉米根系总RNA, 总RNA提取完成后经DNaseI消化, 用于合成cDNA。合成体系为20  $\mu\text{L}$ , 所用试剂盒为Thermo反转录试剂盒(Thermo Scientific, 上海)。采用荧光定量PCR测定玉米碳磷代谢相关基因 *Pht1;2*、*Pht1;6*、*PEPC*、*G3PT*、*TC289*、*MAS1*和AM真菌碳磷代谢相关基因 *GiPT*、*NGT1*、*HXK1b*、*AGM1*、*GlcNAc*、*UAPI*、*CHS1*、*DAC1*、*NAG1*的表达量。定量PCR体系为25  $\mu\text{L}$ , 其中包含12.5  $\mu\text{L}$  SYBR® Premix ExTaq™ (TAKARA Biotechnology, 中国大连), 1.5  $\mu\text{L}$ 稀释5倍的cDNA模板和0.2  $\mu\text{mol}\cdot\text{L}^{-1}$

特异性引物(附录I、附录II)。PCR程序为: (1) 95 °C 10 s; (2) 95 °C 15 s, 60 °C 60 s, 40个循环。60 °C收集荧光数据。溶解曲线分析程序为: 70 °C 10 s, 然后以0.2 °C·s<sup>-1</sup>的升温速率加热到100 °C, 连续收集数据。玉米以*Actin*基因作为内参基因, AM真菌以*EF1 $\beta$* 作为内参基因, 每个样品做3个技术平行。在对照试验中, 分别在反应体系中加入每个RNA样品和水, 以取代cDNA模板, 以此排除基因组DNA污染和引物二聚体的形成。数据分析用2<sup>- $\Delta\Delta\text{Ct}$</sup> 方法(Pfaffl, 2001)。试验所用仪器为Bio-Rad iQ5荧光定量PCR仪(Bio-Rad Laboratories, Hercules, USA), 数据分析软件为Bio-Rad iQ5Optical System Software。

### 1.4.4 数据分析

试验数据用平均值±标准偏差表示。用SPSS 13.0进行双因素方差分析, 分别检验接种处理和磷水平对供体植株或受体植株干质量和磷浓度、植物碳磷代谢相关基因表达的影响。若两因素交互作用显著, 则采用最小显著差异(LSD)法比较所有处理之间的差异显著性; 若二者交互作用不显著, 则按处理分组对数据进行*t*检验。对于菌根侵染率和AM真菌碳磷代谢相关基因表达, 采用*t*检验分析不同磷水平之间的差异显著性。采用Excel 2010生成柱形图。

## 2 结果

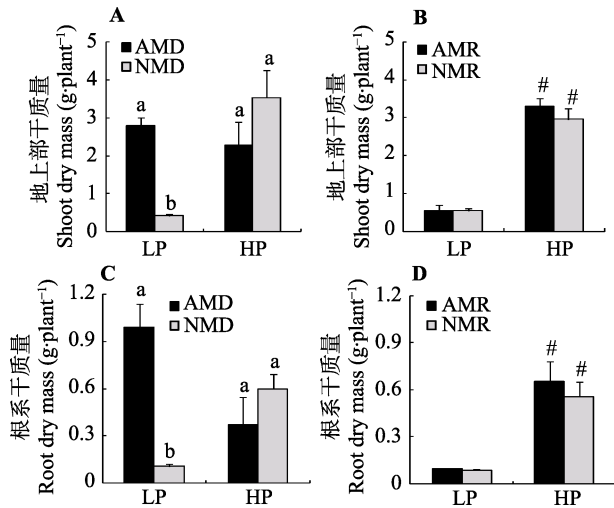
### 2.1 菌根侵染率

试验条件下, 未接种对照玉米根系未检测到菌根侵染迹象, 而接种处理供体玉米根内形成典型的菌根共生结构。低磷(10  $\text{mg}\cdot\text{kg}^{-1}$ )条件下接种处理供体玉米菌根侵染率为68%, 丛枝丰度42%; 高磷(100  $\text{mg}\cdot\text{kg}^{-1}$ )条件下供体玉米菌根侵染率降低至43%, 丛枝丰度23%。菌根侵染率在低磷和高磷处理之间存在显著差异。无论何种试验处理, 受体玉米根系均无菌根侵染。

### 2.2 玉米生长情况

低磷条件下, 接种AM真菌显著提高了供体玉米地上部干质量, 接种处理玉米地上干质量约为不接种处理的5倍。高磷条件下, 接种处理对玉米地上部干重没有显著影响(图2A; 附录III)。无论高磷还是低磷条件下, 供体室接种处理对受体玉米地上部生物量都没有显著影响(图2B; 附录III)。

接种处理对根系生物量的影响与地上部一致。



**图2** 不同磷浓度下接种AM真菌对玉米植株干质量的影响(平均值±标准偏差)。LP和HP分别代表低磷和高磷处理。AMD和NMD分别代表供体植物接种AM真菌和不接种对照处理; AMR和NMR分别代表受体植物受到AM分泌物处理和对照处理。柱形上方标示不同字母代表不同处理间在5%水平有显著性差异。“#”代表在相同接种处理下不同磷水平之间在5%水平差异显著。

**Fig. 2** Effects of mycorrhizal inoculation on maize dry mass under different P levels (mean ± SD). LP and HP refer to low P level ( $10 \text{ mg} \cdot \text{kg}^{-1}$ ) and high P level ( $100 \text{ mg} \cdot \text{kg}^{-1}$ ) respectively. AMD and NMD represent donor plants with and without AM fungus incubation, while AMR and NMR represent receiver plants with and without AM exudates respectively. Different letters above the columns indicate significant difference ( $p < 0.05$ ) between corresponding treatments. # indicates significant difference ( $p < 0.05$ ) between different P levels under the same inoculation treatment.

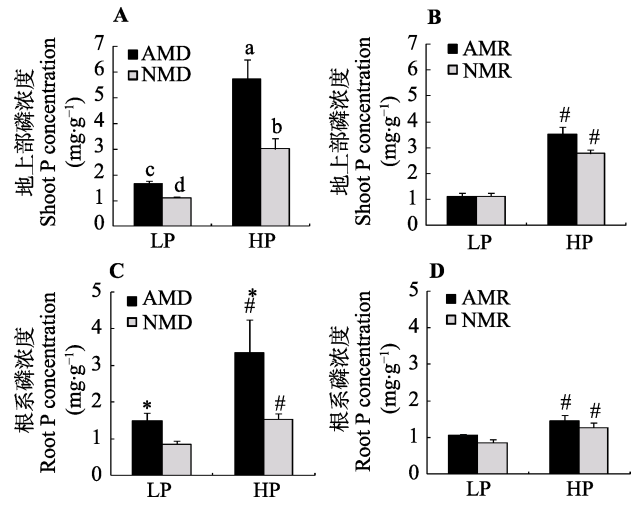
低磷条件下, 供体室接种处理显著提高了供体玉米的根系生物量, 而高磷条件下, 接种对供体玉米的根系生物量没有显著影响(图2C; 附录III)。接种处理对受体室玉米根系生物量总体上没有明显影响(图2D; 附录III)。

接种处理和磷水平对供体玉米植株干质量表现出显著交互作用: 高磷显著提高了未接种对照植物地上部和根系干质量, 但对接种处理植物干质量没有显著影响(图2A、2C; 附录III)。对于受体植物而言, 无论供体室接种与否, 高磷水平均显著提高了植株地上部和根系干质量(图2B、2D; 附录III)。

### 2.3 接种AM真菌对玉米植株磷浓度的影响

无论低磷还是高磷条件下, 接种AM真菌均显著提高了供体玉米地上部磷浓度(图3A; 附录III)。相同磷水平下, 供体室接种处理对受体玉米地上部磷浓度无显著性影响。总体上, 高磷处理显著提高了植株地上部磷浓度(图3B; 附录III)。

接种AM真菌显著提高了供体玉米根系磷浓度,



**图3** 不同磷浓度下接种AM真菌对玉米植株磷浓度的影响(平均值±标准偏差)。LP和HP分别代表低磷和高磷处理。AMD和NMD代表供体植物接种AM真菌和不接种对照处理; AMR和NMR代表供体和受体植物受到AM分泌物处理和对照处理。不同字母代表处理间在5%水平上有显著性差异。“#”代表在相同AM真菌分泌物受体不同磷浓度处理间在5%水平上差异显著。“\*”代表在相同磷浓度处理下AM真菌处理间在5%水平上差异显著。

**Fig. 3** Effects of inoculation with AM fungus on maize P concentrations under different P levels (mean ± SD). LP and HP refer to low P level ( $10 \text{ mg} \cdot \text{kg}^{-1}$ ) and high P level ( $100 \text{ mg} \cdot \text{kg}^{-1}$ ) respectively. AMD and NMD represent donor plants with and without AM fungus incubation, while AMR and NMR represent receiver plants with and without AM exudates respectively. The different letters indicates significant difference ( $p < 0.05$ ) between corresponding treatments. # indicates significant difference ( $p < 0.05$ ) between different P levels under the same inoculation treatment; \* indicates significant difference ( $p < 0.05$ ) between inoculation treatments under the same P level.

低磷条件下接种处理供体玉米根系磷浓度比不接种对照提高了45.6% (图3C; 附录III)。相同磷水平下, AM真菌对受体玉米根系磷浓度无显著性影响(图3D; 附录III)。

### 2.4 不同磷水平下AM真菌碳磷代谢基因表达情况

低磷条件下AM真菌中GlcNAc代谢和磷转运相关基因的表达水平总体显著高于高磷情形。与高磷情形相比, 低磷条件下*GiPT*、*HXK1b*、*CHS1*和*NAG1*表达水平提高了2–3倍(图4)。

### 2.5 供体玉米碳磷代谢相关基因表达情况

低磷条件下, 接种AM真菌显著上调了供体玉米*Pht1;2*、*Pht1;6*、*PEPC*、*TC289*、*G3PT*、*MAS1*基因的表达水平。高磷条件下, 只有*Pht1;2*、*Pht1;6*和*G3PT*的表达量受接种处理上调(图5; 附录III)。

### 2.6 受体玉米碳磷代谢相关基因表达情况

土壤磷水平和接种处理对受体玉米根系的

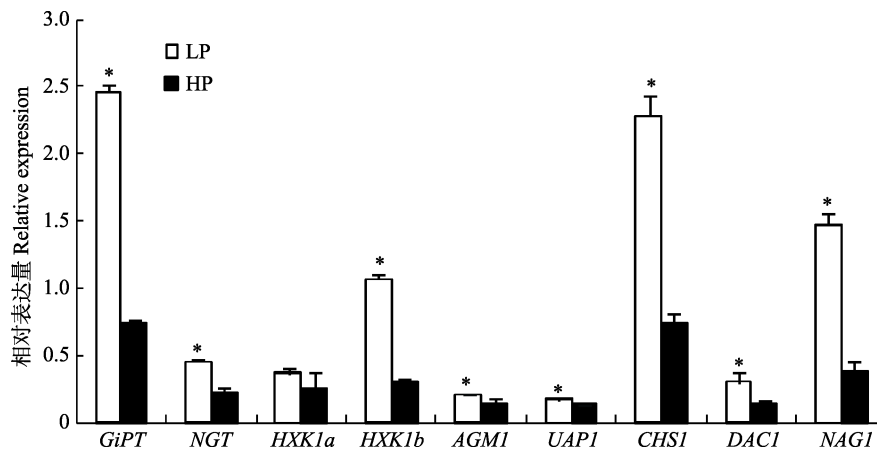


图4 不同磷水平下AM真菌碳磷代谢相关基因表达(平均值±标准偏差)。LP为低磷处理, HP为高磷处理; \*表示不同磷水平之间差异显著( $p < 0.05$ )。 *GiPT*, AM真菌磷转运蛋白基因; *NGT1*, N-乙酰葡萄糖胺(GlcNAc)转运蛋白基因; *HXK1b*, GlcNAc激酶b基因; *AGM1*, GlcNAc磷酸变位酶基因; *UAP1*, UDP-GlcNAc焦磷酸化酶基因; *CHS1*, 几丁质合酶基因; *DAC1*, GlcNAc-6-磷酸去乙酰化酶基因; *NAG1*, 葡萄糖胺-6-磷酸异构酶基因。

**Fig. 4** Expression of AM fungal genes relevant to C and P metabolisms under different P levels (mean  $\pm$  SD). LP refers to low P treatments, HP refers to high P treatments, \* indicates significant difference ( $p < 0.05$ ) between different P levels. *GiPT*, AM fungal P transporter gene; *NGT1*, GlcNAc transporter gene, *HXK1b*, GlcNAc kinase gene; *AGM1*, GlcNAc phosphomutase gene; *UAP1*, UDP GlcNAc pyrophosphorylase gene; *CHS1*, chitin synthase gene; *DAC1*, GlcNAc-6-phosphate deacetylase gene; *NAG1*, glucosamine-6-phosphate isomerase gene.

*Phl1;6*、*G3PT*、*PEPC*、*TC289*、*MAS1*表达具有显著交互作用。高磷处理抑制这些基因的表达, 而供体室接种处理总体上提高了基因表达水平。*Phl1;2*、*Phl1;6*、*G3PT*、*PEPC*、*TC289*、*MAS1*等基因的表达受接种处理显著上调(图6; 附录III)。

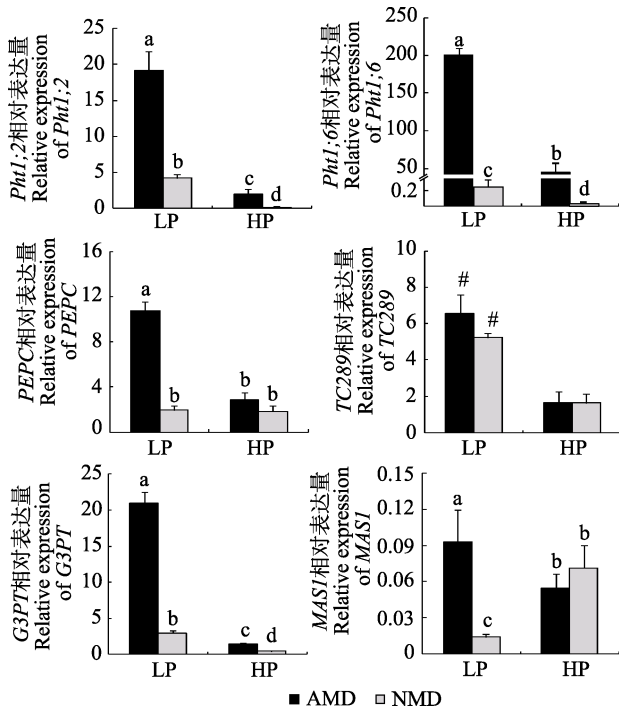
### 3 讨论

在AM共生体系中, AM真菌促进植物对磷的吸收, 植物则为AM真菌提供碳水化合物。在低磷胁迫下, AM真菌不仅通过根外菌丝直接从土壤中获取磷, 还可以调控宿主植物磷转运蛋白基因的表达, 从而帮助植物抵御磷胁迫。本试验采用分室培养系统, 分析不同磷水平下AM共生体系对玉米生长和营养生理的影响。试验结果表明, 低磷条件下AM真菌不仅可以直接改善宿主植物磷营养状况, 还可能通过AM分泌物上调相邻非菌根植物碳磷代谢相关基因的表达, 进而调控植物对低磷胁迫的生理响应。

本试验中, 低磷条件下玉米菌根侵染率显著高于高磷处理, 丛枝丰度也较高, 这与以往的研究结果一致(Gu *et al.*, 2011)。在高磷水平下, 植物会限制碳水化合物向AM真菌的转运, 从而抑制AM共生体发育(Olsson *et al.*, 2006; Nagy *et al.*, 2009)。有研究表明, 磷酸盐能直接抑制*R. intraradices*根内菌丝的分化和丛枝的发育(Harrison *et al.*, 2010)。Breuillin (2010)试验发现, 在10 mmol·L<sup>-1</sup>磷酸盐溶液处理

下, AM真菌丛枝发育不良, 仅有稀疏分枝并过早衰老, 同时根内菌丝的生长和分化也受到抑制。此外, AM真菌中一些关键功能基因, 如磷酸盐转运蛋白基因的表达水平也会被高浓度磷酸盐抑制(Chen *et al.*, 2007)。本实验中, 高磷条件下AM真菌碳磷代谢相关基因*GiPT*、*NGT1*、*HXK1b*、*AGM1*、*UAP1*、*CHS1*、*DAC1*和*NAG1*的表达水平较低, 也表明高磷抑制了AM共生体系的发育和正常功能。相应地, 低磷条件下*GiPT*、*HXK1b*、*CHS1*和*NAG1*基因表达水平显著升高, 则表明低磷胁迫会促进菌根共生体系的建成。

磷作为一种植物必需的营养元素, 在植物生长发育和代谢过程中均起着重要作用(Marschner, 1995)。在本试验中, 低磷条件下接种AM真菌显著促进了玉米生长, 达到和高磷条件下相近的生物量。实验土壤为碱性土(pH值8.69), 有效铁和有效磷较低而不利于植物生长(Tyler, 1999)。以往研究表明*R. irregularis*可以在碱性土壤中与宿主植物形成良好的共生关系。AM不仅可以通过根外菌丝直接吸收土壤中的磷, 还可以分泌草酸并诱导植物根系分泌柠檬酸, 降低土壤的pH值以提高土壤磷的溶解度, 从而促进植物吸收更多的磷(Gardner *et al.*, 1983; Cunningham & Kuiack, 1992)。分子水平的研究表明, 菌根磷转运蛋白基因位于菌根共生信号途径的下游, 在根内皮层细胞形成丛枝之后, 植物根内的菌根特

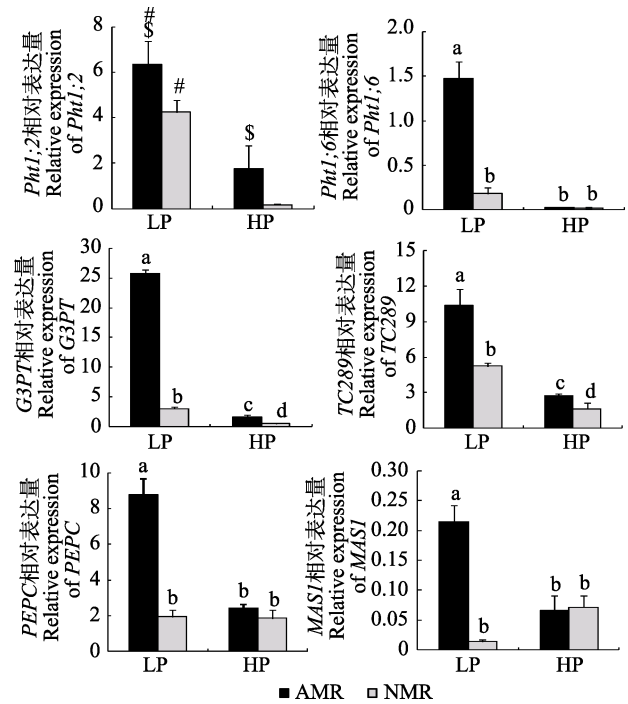


**图5** 不同磷水平下供体玉米碳磷代谢基因表达情况(平均值±标准偏差)。LP和HP分别代表低磷和高磷处理。AMD和NMD代表供体植物接种AM真菌和不接种对照处理。柱形上方标示不同字母代表相应处理之间在5%水平有显著性差异。“#”表示相同接种处理不同磷水平之间在5%水平差异显著。*Pht1;2*, *Pht1;6*, 磷转运蛋白基因; *PEPC*, 磷酸烯醇式丙酮酸羧化酶基因; *G3PT*, 甘油-3-磷酸转运蛋白基因; *TC289*, 无机焦磷酸化酶基因; *MAS1*, 苹果酸合酶基因。

**Fig. 5** Expression of genes relevant to C and P metabolism in maize roots from donor compartment under different P levels (mean ± SD). LP and HP refer to low P level (10 mg·kg<sup>-1</sup>) and high P level (100 mg·kg<sup>-1</sup>) respectively. AMD and NMD represent donor plants with and without AM fungus. Different letters above the columns indicate significant difference ( $p < 0.05$ ) between corresponding treatments. # indicates significant difference ( $p < 0.05$ ) between different P levels. *Pht1;2*, *Pht1;6*, P transporter genes; *PEPC*, phosphoenolpyruvate carboxylase gene; *TC289*, inorganic pyrophosphatase gene; *G3PT*, glycerol-3-phosphate transporter gene; *MAS1*, malate synthase gene.

异性磷转运蛋白被诱导表达并高效地向植物输送磷(Javot *et al.*, 2007; Smith & Read, 2008)。本实验中, 低磷条件下接种AM真菌或AM分泌物都可以显著上调玉米根中*Pht1;2*和*Pht1;6*的表达水平, 尤其是*Pht1;6*基因在低磷条件下受到接种处理的强烈诱导, 表达量与不接种相比提高了近1 000倍。这表明低磷胁迫下AM共生体系特殊的磷吸收转运系统对于改善植物磷营养状况可能具有重要意义。

Carlos等(2008)利用基因芯片研究了磷饥饿对植物基因表达谱的影响, 表明碳代谢、氮代谢、脂代谢和磷代谢基因均有不同响应。本试验中, 低磷环境下AM不仅显著调节了供体植物碳磷代谢相关



**图6** 不同磷水平下受体玉米碳磷代谢相关基因表达情况(平均值±标准偏差)。LP和HP分别代表低磷和高磷处理。AMR和NMR代表供体和受体植物受到AM分泌物处理和对照处理。柱形上方标示不同字母代表相应处理之间在5%水平有显著性差异。“#”表示相同接种处理不同磷水平之间在5%水平差异显著, 而“\$”代表在相同磷水平下不同接种处理之间在5%水平上差异显著。*Pht1;2*, *Pht1;6*, 磷转运蛋白基因; *PEPC*, 磷酸烯醇式丙酮酸羧化酶基因; *G3PT*, 甘油-3-磷酸转运蛋白基因; *TC289*, 无机焦磷酸化酶基因; *MAS1*, 苹果酸合酶基因。

**Fig. 6** Expression of genes relevant to C and P metabolism in maize roots from receiver compartment under different P levels (mean ± SD). LP and HP refer to low P level (10 mg·kg<sup>-1</sup>) and high P level (100 mg·kg<sup>-1</sup>) respectively. AMR and NMR represent receiver plants with and without AM exudates respectively. # indicates significant difference ( $p < 0.05$ ) between different P levels, while \$ indicates significant difference ( $p < 0.05$ ) between inoculation treatments under the same P level. *Pht1;2*, *Pht1;6*, P transporter genes; *PEPC*, phosphoenolpyruvate carboxylase gene; *TC289*, inorganic pyrophosphatase gene; *G3PT*, glycerol-3-phosphate transporter gene; *MAS1*, malate synthase gene.

基因*G3PT*、*PEPC*、*TC289*和*MAS1*的表达, 还上调了受体植物中上述基因的表达。*G3PT*参与糖-磷酸盐/阴离子的逆向转运过程, 低磷胁迫下拟南芥(*Arabidopsis thaliana*)中*G3PT*家族的5个基因均呈现不同程度的上调, 进而调节根的生长或磷酸盐的平衡(Ramaiah *et al.*, 2011)。*PEPC*不仅可以提高植物的光合速率和产量(Fukayama *et al.*, 2003), 还参与促进C<sub>3</sub>植物的三羧酸循环, 为氨基酸合成提供碳骨架(Radchuk *et al.*, 2007)。通过调控*PEPC*表达不仅提高豆类种子的蛋白含量, 也使蛋白质组分发生变化

(Rolletschek *et al.*, 2004)。TC289可以催化无机焦磷酸水解为正磷酸盐,参与合成糖类、核酸和蛋白质等多种代谢途径中的焦磷酸水解过程(Rojas-Beltrán *et al.*, 1999)。AM分泌物对于受体植物碳磷代谢基因的调控,表明AM分泌物很可能通过扩散作用影响到非菌根植物,上调植物碳代谢、磷转运相关基因的表达,最终可能引起植物生理代谢过程的改变,帮助植物抵御低磷胁迫。Kosuta等(2003)首次证明AM真菌可扩散性信号物质的存在,这些信号物质可诱导植物基因*MtEnod11*的表达。Maillet等(2011)获得AM真菌信号物质菌根因子(Myc factor),一种脂质几丁寡糖(Myc-LCOs)的化学结构。有研究表明AM真菌信号分子在诱导植物基因表达(Chabaud *et al.*, 2011)、根内淀粉积累、糖代谢(Gutjahr *et al.*, 2009)和侧根发生(Oláh *et al.*, 2005)过程中作用活跃。限于研究条件,本试验无法区分根系自身分泌物和AM特异分泌物,也未能直接检测AM分泌物及相关信号物质,至于是何种信号物质对植物响应低磷胁迫起到关键调节作用,还需要进一步研究。

综上所述,在植物和AM真菌相互作用的过程中,AM真菌能够通过对植物和自身碳磷代谢基因的调控促进共生体系的建成,进而直接参与和调控宿主植物的磷营养。在AM真菌完全不接触邻体植物的情况下,AM分泌物也可能通过空间扩散作用调控植物基因表达,帮助植物抵御低磷胁迫。在本试验中我们未能分析和测定AM分泌物的动态变化,不能直接建立AM分泌物和植物对低磷胁迫生理响应的直接关联,将来的研究可直接收集或纯化AM分泌物,更为直接地考察AM分泌物的作用,以期全面揭示AM共生体系调控植物磷营养的生理和分子机制。

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附录I 玉米中碳磷代谢相关功能基因定量PCR引物序列

Appendix I The PCR primer sequences for functional genes in maize plants

基因 Gene	正向引物 Forward primer	反向引物 Reverse primer	文献 Reference
Action	GTCCGTGCGTTTCCTTTTGT	AAACCGGCCTTGACCATTCC	Soderlund <i>et al.</i> , 2009
Pht1;2	CCAACTTGCTTGGCTTTATCCT	AGCCTCCCGGACATCTC	Schnable <i>et al.</i> , 2009
Pht1;6	CTACAGCCAGAACCTGACCC	ACATGACGCCCATCAGTAGC	Schnable <i>et al.</i> , 2009
G3PT	TTCACCGCCTGCGTCCTT	TCGCTGGGCTCCTCTTGAG	Carlos <i>et al.</i> , 2008
PEPC	CACGCTGATCCTGACCATGA	TCGCAAACCGAGTATGTATCTT	Carlos <i>et al.</i> , 2008
TC289	CCCTTGGCATGATCTGGAGAT	CCTTGCTGCCCTTGGTAT	Carlos <i>et al.</i> , 2008
MAL1	TGGACGCGTACAACCTCATC	CTGACTCCACTGCCGACAAA	Carlos <i>et al.</i> , 2008

Pht1;2, Pht1;6, 磷转运蛋白基因; PEPC, 磷酸烯醇式丙酮酸羧化酶基因; G3PT, 甘油-3-磷酸转运蛋白基因; TC289, 无机焦磷酸化酶基因; MAL1, 苹果酸合酶基因。  
Pht1;2, Pht1;6, phosphorus transporter genes; PEPC, phosphoenolpyruvate carboxylase gene; G3PT, glycerol-3-phosphate transporter gene; TC289, inorganic pyrophosphatase gene; MAL1, malate synthase gene.

附录II AM真菌中碳磷代谢相关功能基因定量PCR引物序列  
Appendix II The PCR primer sequences for AM fungal genes

基因 Gene	正向引物 Forward primer	反向引物 Reverse primer	文献 Reference
<i>EF1β</i>	CCCATGCAGCTCGATGGTA	TGCCAGGAAGTGAAGAAAATGA	Yoshihiro <i>et al.</i> , 2015
<i>NGT1</i>	TGGCGCAGCACTTTTGTG	CGTTCGGTAGGGTAAGATAACATGA	Yoshihiro <i>et al.</i> , 2015
<i>HXK1a</i>	CGATTGCCAACTGGTATGGA	GCGCAAATTAGTCCCACCTAAG	Yoshihiro <i>et al.</i> , 2015
<i>HXK1b</i>	GGAATCCCAACTGGCAAAGA	ACATTCGTAAATTGTACCTCCAAGA	Yoshihiro <i>et al.</i> , 2015
<i>AGM1</i>	AAAACAATTCGATCTGCTGAAGGT	ATGCTCGTAATTTTCGATTGCT	Yoshihiro <i>et al.</i> , 2015
<i>UAP1</i>	TGAACGCGTCAACCGAATC	CGGTACCGGGAGCAATTTTC	Yoshihiro <i>et al.</i> , 2015
<i>CHS1</i>	CGGCACAATTTAGGGATATAGTA	GGTTCCTCATGAATCAAACCTAGTAA	Yoshihiro <i>et al.</i> , 2015
<i>DAC1</i>	TTTGGAAGAGTTGGTTAATTTGGT	AATACGGTCGCGGACGAA	Yoshihiro <i>et al.</i> , 2015
<i>NAG1</i>	GGCGTTAGCTCTTGCCAAGT	CGCCGAAACGGTAAACATG	Yoshihiro <i>et al.</i> , 2015
<i>GiPT</i>	CTGCTGTTGATTATTGTTGGC	GAACGGTTCCTCATAATAGTG	Maldonado-Mendoza <i>et al.</i> , 2001

*GiPT*, AM真菌磷转运蛋白基因; *NGT1*, N-乙酰葡萄糖胺(GlcNAc)转运蛋白基因; *HXK1b*, GlcNAc激酶b基因; *AGM1*, GlcNAc磷酸变位酶基因; *UAP1*, UDP-GlcNAc焦磷酸化酶基因; *CHS1*, 几丁质合酶基因; *DAC1*, GlcNAc-6-磷酸去乙酰化酶基因; *NAG1*, 葡糖胺-6-磷酸异构酶基因。  
*GiPT*, AM fungal P transporter gene; *NGT1*, GlcNAc transporter gene, *HXK1b*, GlcNAc kinase gene; *AGM1*, GlcNAc phosphomutase gene; *UAP1*, UDP GlcNAc pyrophosphorylase gene; *CHS1*, chitin synthase gene; *DAC1*, GlcNAc-6-phosphate deacetylase gene; *NAG1*, glucosamine-6-phosphate isomerase gene.

附录III 植物干质量、磷含量、碳磷代谢基因表达的双因素方差分析结果  
Appendix III Two-way ANOVA of shoot and root dry mass, P concentrations and expression of genes related to C and P metabolisms as influenced by mycorrhizal inoculation and soil P levels

		地上部干质量 Shoot dry mass	根系干质量 Root dry mass	地上部磷浓度 Shoot P concentration	根系磷浓度 Root P concentration	<i>Pht1;2</i>	<i>Pht1;6</i>	<i>G3PT</i>	<i>PEPC</i>	<i>TC289</i>	<i>MAS1</i>
供体植物 Donor											
接种处理 Inoculation treatment (I)	*		**	*	**	**	**	**	**	ns	**
磷水平 P levels (P)	**		ns	**	**	**	**	**	**	**	ns
交互作用 I × P	**		**	ns	*	**	**	**	**	ns	**
受体植物 Receiver											
接种处理 Inoculation treatment (I)	ns		ns	ns	ns	**	**	**	**	**	**
磷水平 P levels (P)	*		*	*	*	**	**	**	**	**	**
交互作用 I × P	ns		ns	ns	ns	ns	**	**	**	**	**

*Pht1;2*, *Pht1;6*, 磷转运蛋白基因; *PEPC*, 磷酸烯醇式丙酮酸羧化酶基因; *G3PT*, 甘油-3-磷酸转运蛋白基因; *TC289*, 无机焦磷酸化酶基因; *MAS1*, 苹果酸合酶基因。\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; ns, 不显著。  
*Pht1;2*, *Pht1;6*, phosphorus transporter genes; *PEPC*, phosphoenolpyruvate carboxylase gene; *G3PT*, glycerol-3-phosphate transporter gene; *TC289*, inorganic pyrophosphatase gene; *MAS1*, malate synthase gene. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; ns, not significant.

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