Phosphorus acquisition efficiency in arbuscular mycorrhizal maize is correlated with the abundance of root-external hyphae and the accumulation of transcripts encoding PHT1 phosphate transporters

Ruairidh J. H. Sawers[1,2*], Simon F. Svane[3,4], Clement Quan[5], Mette Grønlund[3,4], Barbara Wozniak[2], Eliécer González-Muñoz[1], Ricardo A. Chávez Montes[1], Ivan Baxter[6], Jerome Goudet[7], Iver Jakobsen[3,4] and Uta Paszkowski[2,5]

1. Laboratorio Nacional de Genómica para la Biodiversidad (LANGEBIO), Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Irapuato C.P. 36821, Guanajuato, Mexico.
2. Department of Plant Biology, University of Lausanne, 1014 Lausanne, Switzerland
3. Department of Chemical and Biochemical Engineering, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark
4. Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark
5. Department of Plant Sciences, University Cambridge, Cambridge CB2 3EA, UK
6. USDA-ARS, Donald Danforth Plant Science Center, St. Louis, Missouri, 63132, United States of America
7. Department of Ecology and Evolution, University of Lausanne, 1014 Lausanne, Switzerland

*Author for correspondence:
Ruairidh J. H. Sawers
Laboratorio Nacional de Genómica para la Biodiversidad (LANGEBIO), Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Irapuato C.P. 36821, Guanajuato, Mexico.
Tel. +52 462 1663012
E-mail: rusawers@cinvestav.mx

Word count: 5307 (Introduction: 833, Materials and Methods: 1560, Results: 1760, Discussion: 1091, Acknowledgements: 63).
Figures: 5 (All colour)
Tables: 2
Supporting Information as PDF; Fig.S1 – S5; Table S1 – S2

SUMMARY

- Plant interactions with arbuscular mycorrhizal fungi have long attracted interest for their potential to promote more efficient use of mineral resources in agriculture. Their widespread use, however, remains limited by understanding of the processes that determine the outcome of the symbiosis. In this study, variation in growth response to mycorrhizal inoculation was characterized in a panel of diverse maize lines.

- A panel of thirty maize lines was evaluated with and without inoculation with arbuscular mycorrhizal fungi. The line Oh43 was identified to show superior response and, along with five other reference lines, was characterized in greater detail in a split-compartment system, using ^{33}P to quantify mycorrhizal phosphorus uptake.

- Changes in relative growth between non-inoculated and inoculated plants indicated variation in host capacity to profit from the symbiosis. Shoot phosphate content, abundance of intra-radical and root-external fungal structures, mycorrhizal phosphorus uptake, and accumulation of transcripts encoding plant PHT1 family phosphate transporters varied among lines.

- Larger growth responses in Oh43 were correlated with extensive development of root-external hyphae, accumulation of specific *Pht1* transcripts and a high level of mycorrhizal phosphorus uptake. The data indicate that host genetic factors influence fungal growth strategy with an impact on plant performance.

Key words: arbuscular mycorrhiza, maize, PHT1, phosphorus, root-external hyphae

INTRODUCTION

The rising cost of agricultural inputs and an increasing awareness of the potential negative environmental consequences of their use have resulted in ever greater interest in beneficial crop-microbe interactions and their application (Perez-Montano *et al.*, 2014; Vance, 2014). The most prevalent nutrient-delivering symbiosis is the association of plants with fungi of the phylum *Glomeromycota*, resulting in the formation of arbuscular mycorrhizas (Smith & Read, 2008). More than 80% of extant terrestrial plants establish arbuscular mycorrhizal (AM) symbioses, and this fundamental capacity has been retained in major crop species throughout the processes of domestication and improvement (*e.g.* Koide *et al.*, 1988; Hetrick *et al.*, 1992; Kaeppler *et al.*, 2000; Sawers *et al.*, 2008). Concomitantly, these same crops have retained a conserved molecular machinery required for symbiotic establishment and nutrient exchange (Paszkowski *et al.*, 2002; Gutjahr *et al.*, 2008; Yang *et al.*, 2012; Willman *et al.*, 2013).

The best characterized benefit of AM symbiosis is enhanced plant phosphorus (P) nutrition. The efficiency with which crop plants convert P resources to yield (P Efficiency; PE) can be partitioned between the efficiency of uptake (P Acquisition Efficiency; PAE), and the efficiency of internal use (P Use Efficiency; PUE) (Rose *et al.*, 2011; Veneklaas *et al.*, 2012). Plants take up P in the form of phosphate (Pi), and PAE is typically low in agricultural systems (15-20% of applied P taken up by plants; Syers *et al.*, 2008) as the result of the low mobility of Pi in the soil and the ready formation of a zone of P depletion around the root. By extending beyond the P depletion zone, the root-external hyphae of AM fungi increase the extent of soil foraging and P uptake (Bucher, 2007). Physiological studies have demonstrated that Pi uptake via AM fungi is a distinct functional alternative to direct uptake by the plant (Smith *et al.*, 2003; Bucher, 2007), and, in a field setting, the majority of the Pi taken up by a

plant may be acquired via the mycorrhizal route (Schweiger and Jakobsen, 1999; Smith *et al.*, 2003; Yang *et al.*, 2012). Molecular analyses support the distinction between mycorrhizal and direct P uptake, members of the plant PHT1 P transporter family having been identified to play roles specific to the two pathways (Bucher, 2007). Where plants are competent to host AM fungi, there is at least one family member acting predominantly, or exclusively, during AM symbiosis (ZmPT6 in maize and variously named orthologs in other species; here, the short form PT is used to refer to all PHT1 genes and proteins; Rausch *et al.*, 2001; Harrison *et al.*, 2002; Paszkowski *et al.*, 2002; Glassop *et al.*, 2005; Nagy *et al.*, 2005; Maeda *et al.*, 2006; Caesar *et al.*, 2014; Walder *et al.*, 2015). In *Medicago truncatula*, the ZmPT6 ortholog MtPT4 has been localized to the peri-arbuscular membrane (Harrison *et al.*, 2002; Kobae & Hata, 2010; Pumplin *et al.*, 2012), consistent with a function as the principal route of P uptake from fungus to plant.

While AM symbiosis can increase PAE and also provide enhanced tolerance to a range of abiotic and biotic stresses (Smith & Read, 2008), such benefits are not provided without cost. The plant host must supply carbohydrates to support fungal growth, diverting photosynthetically fixed carbon away from primary productivity. Ultimately, the outcome, in terms of the impact on plant growth and fitness (or yield in crop plants), will depend not only on the specific plant-fungus combination (Walder *et al.*, 2012) but on the requirements and limitations imposed by any given environment (Janos, 2007). In high-input agricultural systems, AM symbiosis may provide no benefit to yield, or even be detrimental (Grace *et al.*, 2009), and it has been hypothesized that plant breeding may have promoted a weakening of the mutualism in modern elite cultivars (Hetrick *et al.*, 1992, 1996). Drawing firm conclusions as to impact of AM symbiosis, however, is difficult. Mycorrhizal response

(defined here as MR = M – NC, where M is the trait value for colonized plants and NC the trait value for non-colonized plants) confounds plant adaptation to a given set of conditions and capacity to benefit from the symbiosis *per se* (discussed in Sawers *et al*., 2010). To emphasize this distinction, the term dependence has been defined as the nutrient requirement of a particular variety to attain a certain threshold level of performance (Janos *et al.*, 2007). In practice, poorly adapted (*i.e.* highly dependent) varieties will typically show a large performance increase following AM colonization (e.g. Hetrick *et al*., 1992; Kaeppler *et al*., 2000; Paszkowski & Boller, 2002): such varieties, although highly responsive, are clearly of little agronomic interest. The question remains as to whether certain varieties derive greater benefit from AM symbioses *per se* than others and to what extent plant breeding can optimize these interactions for agricultural systems (Sawers *et al.*, 2008; Fester & Sawers, 2011).

The objective of this study was to identify maize line(s) highly responsive to mycorrhiza inoculation, conducting detailed physiological analysis to demonstrate greater benefit in selected line(s) when colonized.

MATERIALS AND METHODS

Evaluation of response to *Funneliformis mosseae* in maize diversity panel

A panel of 30 diverse maize lines, comprising the 26 diverse inbred founders of the maize NAM population (McMullen *et al*., 2009), Pa36 (a line tolerant of low P availability; Kaeppler *et al.*, 2000), and the broadly used reference lines B73 and W22, and W64A (a line used previously for study of AM symbiosis; Paszkowski *et al.*, 2006), was evaluated with (M) or without (NC) inoculation with *F. mosseae* (isolate number 12, European Bank of Glomales, http://www.kent.ac.uk/bio/beg/). Plants were grown in 1 litre pots in a mixture of

1:10 loam: quartz sand, as previously described (Sawers *et al*., 2010). The greenhouse was maintained at 28°C day/night temperature with a 12-h light period, including supplementary light when required. Plants were fertilized three times per week with 100 ml of modified Hoagland solution (Hoagland and Broyer, 1936) containing 10% (100μM) of the standard concentration of KH_2PO_4, the potassium concentration being maintained by addition of KCl. A total of 1200 plants (30 genotypes x 2 treatments x 20 replicates) were grown in a complete block design, over five separate plantings, at the University of Lausanne, Switzerland. Plants were harvest 8 weeks and shoot dry weight measured (SDW). For two plantings (corresponding to six complete blocks) roots were collected also, and stained to confirm efficacy of the fungal inoculum as previously described (Gutjahr *et al.*, 2008). Prior to analysis of SDW, data was adjusted to account for differences among plantings. The main effect of inoculation (mycorrhiza response (MR) = M – NC; Sawers *et al*., 2010) was estimated as the difference in means of M of NC groups and shown to be significant using a *t*-test ($p < 0.001$). MR was estimated similarly for each genotype and a 95% *t*-interval calculated for the difference M – NC. ANOVA (R statistics; R Core Team, 2016) was used to compare SDW among the sixty line/treatment combinations ($p < 0.001$) and means groups were calculated using least significant difference (R statistics agricolae::LSD.test; de Mendiburu, 2016).

Determination of elemental concentration by ICP-MS analysis

Weighed tissue samples were digested in 2.5mL concentrated nitric acid (AR Select Grade, VWR) with internal standard added (20ppb In, BDH Aristar Plus). Sample digestion and dilution was carried out as described previously (Ziegler *et al.*, 2013). Elemental

94 concentrations of B, Na, Mg, Al, P, S, K, Ca, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Mo, and
95 Cd were measured using an Elan 6000 DRC-e mass spectrometer (Perkin-Elmer SCIEX)
96 connected to a PFA microflow nebulizer (Elemental Scientific) and Apex HF desolvator
97 (Elemental Scientific). A control solution was run every tenth sample to correct for machine
98 drift both during a single run and between runs. Statistical analysis was conducted with R
99 statistics (see above).

100 **Characterization of AM phosphorus uptake in six selected lines**

101 Six maize lines, selected on the basis of pre-screening of a panel of 30 lines, were evaluated
102 at the Technical University of Denmark. Plants were grown in 2.4 L PVC tubes in accordance
103 with Smith *et al.* (2003). The growth medium (hereafter referred to as soil) was a 1:1 (w:w)
104 mixture of quartz sand and γ-irradiated soil (15 kGy) that had basal nutrients (Pearson &
105 Jakobsen, 1993) and KH_2PO_4 at nil, 15 or 90 mg P kg^{-1} added in solution and carefully
106 mixed into the soil. The P additions resulted in a bicarbonate-extractable P content of 7.9,
107 15.5 or 53.3 mg P kg^{-1} (Olsen *et al.*, 1954). The root/hyphae compartment (RHC) contained
108 2750 g soil and the hyphae compartment (HC) was a small plastic vial placed in the middle of
109 the RHC. The HC contained 55 g of ^{33}P-labeled soil (5 kBq g^{-1}) and was lined with a 25 μm
110 nylon mesh at both ends to prevent root in-growth. Seven weeks later, bicarbonate extracts of
111 HC had a specific activity (SA = $^{33}P/^{31}P$) of 144.7, 79.9 or 29.4 kBq mg^{-1} P, corresponding to
112 no addition or addition of 15 or 90 mg P kg^{-1}. Each maize line was grown in 8 replicate pots
113 in half of which 140 g dry soil-root inoculum of *Rhizophagus irregularis* BEG87 was
114 thoroughly mixed into the growth medium. Filtered BEG87 inoculum leachings were added
115 to all pots as an attempt to establish the same soil microbial community (Pearson & Jakobsen,
116 1993). Two pre-germinated seeds were planted in each pot and thinned to one at the two leaf

stage. Plants were maintained under controlled conditions (12 hour day length at 500 µmol m^{-2} sec^{-1}, 28/20°C day/night and 60 % relative humidity) and watered daily by weight to 70% of the water holding capacity. Plants received supplemental N (NH_4NO_3), Mg and S ($MgSO_4^{2-}$) periodically to additionally provide 375 mg N, 15 mg Mg and 20 mg S per pot. Shoots were harvested at growth stage 51 (BBCH scale; tassel emergence at the top of the stem), oven dried to constant weight at 70°C and dry weights were recorded. Root systems were carefully washed clean using a pressurized water jet and a fine mesh to collect fine root pieces. Roots were blotted dry and total fresh weight (FW) was recorded. Subsamples were taken for root length/colonization measurement (1.5g FW, stored in 50% EtOH) and RNA extraction (1g, flash-frozen in liquid nitrogen). Dried and ground shoot and root samples were oxidized in a 4:1 mixture (v:v) of 65% nitric:70% perchloric acids, and total P was determined by the molybdate blue method using AutoAnalyzer 3 (Bran+Luebbe, Norderstedt, Germany). The ^{33}P in shoot tissue was determined in the same digests in a Packard TR 1900 liquid scintillation counter (PerkinElmer, Waltham, MA, USA). Specific activities of ^{33}P in shoots and the specific activities in bicarbonate extracts of HC soil were used to estimate the amount of P taken up from the HC in accordance with Smith et al. (2004). The abundance of total fungal structures (hyphae, arbuscules or vesicles) or arbuscules specifically was evaluated microscopically as percentage of root length using the grid-line intersect method (Newman, 1966) after clearing and staining (Kormanik & McGraw, 1982). The length of hyphae in HC soil was measured by an aqueous extraction and membrane filter technique (Jakobsen *et al.*, 1992) with the modification that the stained filters were mounted on slides using immersion oil instead of lactoglycerol-trypan blue solution in order to facilitate discrimination of AM and non-AM fungal hyphae. Statistical analysis was conducted with R statistics (see above).

140 **Bioinformatic identification of maize *Pht* genes**

141 To identify a complete set of putative PHT1 encoding genes in maize, the *Saccharomyces*
142 *cerevisiae* PHO84 protein (Uniprot id P25297) was used as a BlastP query (Altschul *et al.*,
143 1990) to search the primary transcript predicted protein sequences from version 6a of the
144 annotated B73 maize genome (Schnable *et al.*, 2009), obtained from Phytozome 10
145 (Goodstein *et al.* 2012). Using a cut-off E-value of $1e^{-54}$, 13 gene-models were retrieved and
146 aligned using MUSCLE (Edgar, 2004). All 13 sequences contained the conserved
147 GGDYPLSATIxSE motif in helix 4 reported previously to be present in PHT proteins
148 (Karandashov & Bucher, 2005). The resulting block-alignment file was converted to
149 Stockholm 1.0 format, and used as input to hmmbuild (HMMER suite version 3.1b2) to
150 search (hmmsearch) the maize primary transcript predicted protein sequences for additional
151 PHT1 proteins. 35 new protein sequences were identified based on an inclusion threshold of
152 E-value <0.01. None of these additional sequences, however, contained the conserved
153 GGDYPLSATIxSE motif and consequently there were not considered to be authentic PHT1
154 proteins. The final list of 13 maize PHT1 genes was consistent with the report of Liu *et al.*,
155 2016.

156 **Analysis of *ZmPt* transcript accumulation**

157 Gene specific primers for real time PCR analysis of *ZmPt* transcript accumulation were
158 designed and successfully optimized for twelve of thirteen annotated genes (Table S2). All
159 primer sets were used in a preliminary characterization of B73 plants, and five sets (*Pt3*, *Pt1*,
160 *Pt6*, *Pt4*, *Pt5*,) selected on the basis of accumulation pattern for analysis of accumulation in
161 the roots of six maize lines grown in the P uptake experiment. Samples were prepared using
162 the LightCycler 480 SYBR green I master mix kit (Roche; Mannheim, Germany) before

analysis on a Roche 480 LightCycler. Each biological sample was analysed as three technical replicates. Three water controls were used for each gene tested. qRT-PCR expression and melting curves were calculated using the LightCycler 480 software (Roche, Version 1.5.9, Mannheim, Germany). Samples were normalized to the geometric mean of expression levels of 3 constitutive genes (*GAPDH, Cyclophilin2, ß-actin*) as described earlier (Gutjahr *et al.*, 2008). In addition to phosphate transporters were analysed together with an AM specific marker gene *ZmAm3*, ortholog of *OsAM3* (Gutjahr *et al.*, 2008) and a *Rhizophagus irregularis* elongation factor gene (Sokolski *et al.*, 2010). Statistical analysis was conducted with R statistics (see above).

Principal component analysis of combined growth, physiology and molecular data sets

Pairwise correlations were calculated for a matrix of growth, physiological and molecular data obtained from six selected lines as described above (shoot dry weight, shoot P content, root dry weight, root P content, total colonization, arbuscule abundance, length of root-external hyphae, P uptake from the hyphal compartment, accumulation of *Pt3*, *Pt1*, *Pt6*, *Pt4*, *Pt5*, *Am3* and *RiEF* transcripts) using R statistics Hmisc::rcorr (Harrel FE, 2016)and the results visualized using R statistics corrplot::corrplot (Wei & Simko, 2016). Principal component analysis (PCA) was performed with R statistics ade4::dudi.pca (Dray & Dufour, 2007) using centered and scaled data, and the results visualized with ade4::scatter.

RESULTS

The line Oh43 exhibits typical dependence but high mycorrhizal responsiveness

To identify lines showing a typical level of dependence (defined here as growth in the absence of inoculation) but high mycorrhiza response (MR), a panel of thirty diverse maize lines (the parents of the maize Nested Association Mapping population and a number of additional lines; see Materials and Methods) was evaluated with (M) or without (NC) inoculation with the fungus *Funneliformis mosseae*. The composition of the diversity panel was determined previously to maximize sampling of genetic diversity from global maize breeding germplasm (McMullen *et al.*, 2009). Use of this panel was designed to provide a context to evaluate the relative performance of any given line, whether inoculated or not.

A total of 1200 plants (30 genotypes x 2 treatments x 20 replicates) were grown in a complete block design, using 1L pots, in P limiting conditions, for a period of eight weeks after emergence (V8 stage). Roots were harvested from 30% of the total experiment and fungal structures by microscopic inspection to confirm the efficacy of inoculation (*Supporting Information*). NC plants were confirmed to be free of fungal structures, while in M plants, the level of colonization was generally high, with a mean of 57% ±0.7% (95% interval for proportion) of root positions examined containing at least one type of fungal structure (hyphae, arbuscules or vesicles), although a broad range of colonization was observed (5% - 98%). For all experimental blocks, the aerial portion of the plants was dried and shoot dry weight (SDW; g) determined (Table 1). Overall, the evaluated lines showed greater growth when colonized by *F. mosseae* (Fig. S1), with a significant ($p < 0.001$) increase in mean SDW from 1.05g in NC plants to 2.16 g in M plants, equating to panel-wide MR of 1.1 ±0.08 g (MR = M – NC; 95% interval for difference in means). At the level of the individual lines, all showed a significant increase in SDW following inoculation (Fig. 1a; Table 1). The most responsive line Oh43 showed a significantly greater MR than the least

responsive line Mo18W (MR_{Oh43} = 1.85 ±0.54 g; MR_{Mo18W} = 0.72 ±0.28 g; 95% t-intervals for MR do not overlap). The contrast in outcome between Oh43 and Mo18W was reflected by rank-changing shifts in SDW relative to other lines in the panel between NC and M conditions: Oh43 and Mo18W were similar to each other, and typical of the panel as a whole, when not inoculated, but differed to each other and were outlying when inoculated (Fig. 1a, b; Table 1; LSD, $\alpha = 0.05$). The line Oh43, by exhibiting typical dependence but high MR, fulfilled the criteria established to identify superior capacity to benefit from AM symbiosis.

Evaluation was conducted under P limiting conditions, and it was expected that variation in growth would be driven largely by differences in P efficiency. Leaf P concentration (ppm) was quantified using inductively coupled plasma-mass spectroscopy (Table S1) and correlated positively with SDW in M plants (Fig. 1c; $p<0.01$, $r^2 = 0.24$). Leaf P concentration was similar in Mo18W and Oh43 when non-colonized but different when plants were inoculated (Fig. 1a, b; Table S1; LSD, $\alpha = 0.05$), mirroring SDW, and suggesting the difference in MR between the two lines to be the result of greater PAE in Oh43 when colonized.

In summary, evaluation of shoot dry weight and P concentration in the diversity panel identified the line Oh43 to be highly responsive to inoculation with *F. mosseae* on the basis of functional differences in the symbiosis.

High mycorrhizal responsiveness in Oh43 is correlated with abundant root-external hyphae

To characterize further the mechanistic basis of MR in Oh43, the line was evaluated in a previously described split-compartment system, separating root/hyphae (RHC) and hyphae

231 compartments (HC) for direct quantification of AM mediated P uptake through ^{33}P labeling
232 (Smith *et al.*, 2003). Although it was not feasible to evaluate the complete panel in this more
233 detailed study, five additional lines were included to provide a context for interpretation of
234 the data, namely the low MR line Mo18W, the widely used reference lines B73 and Mo17,
235 and HP301 and Pa36, the lines that showed the highest and lowest NC SDW, respectively, in
236 the panel evaluation (Fig. 1; Table 1). Eight replicates of the six lines were evaluated at three
237 P levels (low = 7.9 mg P kg^{-1}; medium = 15.5 mg P kg^{-1}; high = 53.2 mg P kg^{-1}), with (M) or
238 without (NC) inoculation with *Rhizophagus irregularis*, a fungal species that has been
239 previously used with success in this system. Given that plants may respond differently to
240 different fungal species and strains (*e.g.* Angelard *et al.*, 2010), the following discussion and
241 further conclusions are confined to this *R. irregularis* experiment.
242 Plants were harvested at tassel emergence, and samples taken for measurement of
243 SDW, P content, and abundance of fungal structures. In addition, soil was collected from the
244 HC for quantification or root-external hyphae. In all lines, SDW increased with greater P
245 addition, irrespective of inoculation status (Fig. 2; Table2). In contrast, MR decreased from
246 positive to negative with increasing P availability (mean of six lines: low P, mean MR =
247 9.56g; medium P, mean MR = 3.95g, high P, mean MR = -1.57g), the ranking of the lines
248 with respect to MR changing also. At low P, Oh43 was more responsive than the other lines,
249 consistent with the results of the *Funneliformis mosseae* experiment, and remained highly
250 responsive at medium P, although exhibiting negative MR at high P (Fig. 2). As observed
251 previously in the *F. mosseae* experiment, SDW was correlated with shoot P content (Fig. 2).
252 Under low P, shoot P content in Oh43 was typical in non-inoculated plants, but high when
253 plants were colonized. Quantification of P uptake from the HC indicated that Oh43 was

indeed obtaining more P via the mycorrhizal pathway under low P than the other lines (Table 2). When the roots were examined, however, Oh43 was not more heavily colonized; indeed, the proportion of root length containing arbuscules was marginally lower (Fig. 3a). was observed to be significantly greater than in the other lines (Fig. 3b; Table 2). Among the six lines, and across all three levels of P availability, a clear relationship was observed between P uptake from the HC and the length of root-external hyphae (Fig. 3b). At high P, the growth response was apparently saturated in the lines B73 and Pa36, which attained their maximum growth irrespective of AM inoculation, although P content was lower in the shoots of M plants than NC plants (*i.e.* PUE was greater in M plants). Interestingly, although inoculated B73 and Pa36 plants showed equivalent growth, P uptake and abundance of intra-radical fungal structures at high P, the length of root-external hyphae was greater in B73. Indeed, B73 supported a generally high level of root-external hyphae: second only to Oh43 at low P, and greater than all other lines at high P. Collectively, these data indicate that Oh43 is highly responsive to inoculation with *R. irregularis* at low P availability, with concomitant extensive development of root-external hyphae and high PAE in inoculated plants.

Accumulation of *ZmPt* transcripts reflects functional differences among AM plants

To obtain further evidence that MR variation was linked to functional differences among M plants, accumulation of plant PHT1 phosphate transporter encoding transcripts (Fig. S3; Liu *et al*., 2016) was quantified in the roots of plants harvested from the *Rhizophagus irregularis* experiment. A preliminary analysis using the line B73 (Fig. S4) and previous reports (Fig. S6; Liu *et al*., 2016) were used to select the transcripts *ZmPt1*, *ZmPt3*, *ZmPt4*, *ZmPt5* and *ZmPt6* as being the most informative (based on tissue-specific expression and response to

277 inoculation) for quantification in the six lines (Fig. 4). Across the six lines, the accumulation
278 of *ZmPT* transcripts with respect to tissue type, inoculation with AM fungi and P availability
279 was similar to that in B73 (Fig. S5). Within broad qualitative trends, however, quantitative
280 differences were observed (Fig. 4).

281 To investigate patterns of variation in the accumulation of *ZmPt* transcripts, and to
282 integrate transcript accumulation data with plant growth and physiological measurements, a
283 principal component (PC) analysis was performed using the complete data set for M plants
284 under low P (Fig. 5). In addition, pairwise correlations were calculated directly among all
285 measurements (Fig. S5). The first two PCs captured 76% of the trait variation and well
286 separated the six lines (Fig. 5). As expected from the previous analysis, plant dry weight was
287 associated in the PC space with abundance of root-external hyphae, P uptake from the hyphae
288 compartment (PHC) and shoot P content (Fig.5, lower right quadrant). Accumulation of
289 *ZmPt1* and *ZmPt3* transcripts was associated with the same region of the PC space, and
290 accumulation of *ZmPt3* transcripts was found to be positively correlated with root dry weight
291 (RDW; $p < 0.05$) , root P content ($p < 0.1$) and PHC ($p < 0.1$). Abundance of intra-radical
292 fungal structures (%Col) was opposite to that of root-external hyphae in the PC space (Fig. 5,
293 upper left compared with lower right quadrants), and a negative correlation was found
294 between % Col and the length of root-external hyphae, suggesting a possible trade-off in the
295 pattern of fungal growth. This correlation was, however, not significant at the 0.1 level
296 indicating that this relationship did not hold for all lines. Accumulation of the marker
297 transcripts *Am3* and *RiEF* was associated with accumulation of *ZmPt4, ZmPt5* and *ZmPt6*,
298 and root P content (Fig. 5, upper right quadrant). Interestingly, although accumulation of
299 *ZmPt6* strongly differentiated NC and M plants (Fig. S5), among these six lines, when

inoculated, no correlation was observed between *ZmPt6* accumulation and arbuscule abundance. Placing the lines on the PC space, Oh43 was separated from other lines on the basis of PC2 (Fig. 5, lower right quadrant), associated with shoot P, length of root-external hyphae, PHC and to a lesser extent accumulation of *ZmPt4* and *ZmPt5*. The lines Mo18W and Hp301 were associated with low biomass and low levels of *ZmPt* transcript accumulation, but high levels of intra-radical colonization (Fig. 5, upper left quadrant). Mo17 was distinct in accumulating high levels of AM associated transcripts, although with no associated increase in colonization, development of root-external hyphae or MR. Taken as a whole, physiological and molecular data support the interpretation that superior MR in Oh43 results from the nature of the fungal-plant interaction, and is not an artifact resulting from a high level of dependence.

DISCUSSION

Data presented in this study reveal genetic variation in the capacity of maize varieties to profit from AM symbiosis, beyond differences in plant dependence (Janos 2007; Sawers *et al*., 2008, 2010). Evaluation of the relative growth of thirty highly diverse lines (McMullen *et al*., 2009) with and without inoculation with *Funneliformis mosseae* distinguished those that were highly responsive on the basis of poor performance in the absence of symbiosis (*i.e.* highly dependent) from those that benefited more from the symbiosis *per se*. The line Oh43 was selected as an example of the latter. Support for this initial interpretation was obtained by detailed physiological and molecular characterization linking superior responsiveness of Oh43 to *Rhizophagus irregularis* with enhanced PAE in mycorrhizal plants and a greater abundance of root-external hyphae.

Phosphorus limitation of plant growth results primarily from the low mobility of P in the soil. In this study, it was observed that increased plant growth in AM colonized plants was accompanied by greater shoot P content. Once P has reached the root-surface or been delivered to the peri-arbuscular space, subsequent uptake by plant PHT1 transporters is not predicted to limit PAE (Bucher, 2007). As is consistent, it was neither the extent of intra-radical colonization nor the accumulation of *ZmPt6* transcripts (predicted to encode the major peri-arbuscular membrane associated PT transporter) that showed the greatest correlation with P uptake, but the abundance of root-external hyphae. This generally supports previous studies (*e.g.* Schweiger & Jakobsen 1999, Jakobsen *et al.*, 2001, Yao *et al.*, 2001, Schnepf *et al.*, 2008), including an evaluation of diverse fungal isolates used to inoculate a common plant host, which found a similar correlation between fungal P uptake and hyphal length (Munkvold *et al.*, 2004). Although a further report characterizing variation in mycorrhiza response among four Chinese maize varieties did not reveal a clear relationship between P uptake and the length of root-external hyphae (Chu *et al.*, 2013), this may reflect the specific genotypes evaluated, or the fact that the contribution of mycorrhizal P uptake was not directly quantified. A potential trade-off was observed between the abundance of intra-radical and root-external fungal structures, the balance of which is apparently influenced by plant genetic factors. Given the importance of hyphal abundance to PAE, the data suggest that quantification of intra-radical structures alone is not predictive of P uptake via the mycorrhizal pathway or growth response.

Prior physiological characterization has demonstrated that mycorrhizal P uptake is not a simple addition to direct P uptake, but may represent a functional alternative: in the extreme case, a colonized plant may obtain nearly all of its P requirement via the AM

345 pathway, whether as a result of down regulation of the direct pathway or owing to a greater
346 efficiency of fungal P foraging compared with that of plant roots (Smith *et al.*, 2003; Schnepf
347 *et al.*, 2008). Furthermore, the mycorrhizal pathway may remain important at higher P
348 availability, even when MR itself is small, or even negative. Following inoculation with *R.*
349 *irregularis*, the absolute quantity of P obtained via the AM pathway was greater at high P
350 than at low P, even though the abundance of intra-radical and root external fungal structures
351 was lower, presumably as the result of increased Pi in solution as the capacity of the soil to
352 adsorb P was saturated. Collectively, these data illustrate the complexity of determining
353 symbiotic outcome, and the range of plasticity among just six lines, with respect to just a
354 single environmental variable.

355 Transcriptome profiling has identified rice marker genes whose transcript
356 accumulation correlates well with the establishment and development of AM symbiosis,
357 differentiating colonized from non-colonized plants (*e.g.* Guimil *et al*., 2005; Gutjahr *et al*.,
358 2015). In this study, transcripts encoding PHT1 phosphate transporters were quantified
359 primarily not to distinguish NC and M plants in general, but to investigate differences among
360 the different lines when colonized. Overall, AM colonization was positively correlated with
361 the accumulation of transcripts encoded by *Pt6*, and to a lesser extent those encoded by *Pt4*,
362 *Pt5* and *Pt2*, consistent with previous reports (Nagy *et al.*, 2006; Willmann *et al.*, 2013; Liu
363 *et al*., 2016). Variation in the accumulation of the *ZmPt6* transcript, however, was largely
364 independent of differences in growth response to *R. irregularis*, although arbuscule
365 abundance itself was also a poor predictor of MR among the six lines evaluated in this study.
366 These observations are consistent with the interpretation that P transfer at the peri-arbuscular
367 interface is non-limiting, with respect to either arbuscule abundance or the concentration of

368 PT6 proteins in the peri-arbuscular membrane. PT6 protein, however, has been reported also
369 to regulate developmental responses to P limitation (Volpe *et al*., 2016), and variation in
370 *ZmPt6* accumulation may have additional significance beyond P transfer. A number of
371 additional maize *ZmPt* transcripts responded to AM inoculation, although they were less
372 abundant than those encoded by *ZmPt6*. Significantly, at low P, a mild positive correlation
373 was observed between accumulation of *ZmPt4* and *ZmPt5* transcripts and shoot biomass
374 among colonized plants, indicating a role in the regulation of the symbiosis. Accumulation of
375 *Pt1* and *Pt3* transcripts, although generally lower in M than NC plants, was positively
376 correlated with root and shoot dry weight among M lines. Accumulation of *ZmPt1* was
377 positively correlated also with P uptake from the hyphal compartment. Interestingly, the
378 correlation was stronger with dry weight than P content, indicating this may be more than a
379 secondary effect of differences in P accumulation. These observations, along with previous
380 characterization of *pt11* and *pt13* mutants in rice (Yang *et al*., 2012), suggest a role for PHT1
381 proteins not only in P uptake but in the fine tuning of cost-benefit in AM symbioses.

382 Previous characterization of variation in MR has placed an emphasis on the
383 development of intra-radical fungal structures, and marker transcripts have been identified
384 allowing molecular-based quantification of intra-radical colonization. In this study, it was
385 observed that variation in the abundance of root-external hyphae was better correlated than
386 levels of intra-radical colonization with plant growth response to inoculation with *R.*
387 *irregularis*. Although accumulation of the well characterized *ZmPt6* transcript was not
388 predictive of the abundance of extra-radical hyphae, correlations were observed between
389 transcripts encoded by other *ZmPt* genes, abundance of extra-radical hyphae and mycorrhiza
390 response. The identification of such variation, coupled with the availability of populations for

391 quantitative trait loci mapping (McMullen *et al.*, 2009), opens up the possibility to
392 characterize the genetic basis of host effects on the development of extra-radical hyphae, and
393 to develop molecular breeding strategies to target this important, but hard to evaluate,
394 component of the outcome of mycorrhizal symbiosis.

395

ACKNOWLEDGEMENTS

This work was supported by the Swiss National Science Foundation 'professeur boursier' grants PP00A-110874, PP00P3-130704, by the Gatsby Charitable Foundation grant RG60824, by The Danish Council for Independent Research, Technology and Production Sciences grant 0602-01412B and by the Mexican National Council of Science and Technology (CONACYT) grant CB2012-151947. We thank Mesfin Nigussie Gebreselassie and Matthias Mueller for assistance with plant growth and evaluation.

AUTHOR CONTRIBUTION

Diversity screen: RS, BW, UP. Ionomic analysis: IB. Physiological characterization of phosphate uptake in selected lines**:** SS, MG, IJ. Identification of *Pht1* genes and phylogenetic analysis: EGM, RCM, RS. Quantification of transcript accumulation: CQ, SS. Statistical analysis: JG, RS. Experimental design: UP, IJ, RS. All authors contributed to data interpretation and writing of the manuscript.

Table 1. Shoot dry weight of 30 maize lines grown with or without inoculation with *Funneliformis mosseae.* Shoot dry weight of 8 week-old non-colonized (NC; *n* = sample size) and mycorrhizal (M; inoculated with *F. mosseae*; *n* = sample size) plants. Means groups based on least significant difference (LSD) were calculated separately for NC and M plants at $\alpha = 0.05$. Mycorrhiza response (MR) was calculated as M-NC, numbers in parentheses give a 95% confidence interval (CI) for the difference in means.

Line	NC (g)	*n*	NC LSD	M (g)	*n*	M LSD	MR (95% CI)
Oh43	0.96	20	ghijklm	2.81	20	ab	1.85 (1.31 ,2.38)
CML322	1.13	18	efghi	2.59	17	abcde	1.47 (0.8 ,2.14)
HP301	0.44	18	n	1.82	18	ij	1.37 (0.91 ,1.84)
A188	0.98	13	fghijklm	2.3	14	bcdefghi	1.32 (1.04 ,1.61)
W64	1.02	20	fghijkl	2.34	19	bcdefgh	1.32 (0.91 ,1.73)
B97	0.74	20	m	2.04	20	fghij	1.31 (0.89 ,1.73)
NC350	0.86	18	jklm	2.17	18	defghi	1.31 (0.8 ,1.81)
M162W	1	17	fghijkl	2.23	14	cdefghi	1.23 (0.86 ,1.61)
P39GB	0.87	18	ijklm	2.11	19	efghij	1.23 (0.79 ,1.67)
Pa36	1.67	15	a	2.91	15	a	1.23 (0.71 ,1.76)
Ms71	1.4	19	bcd	2.62	20	abcd	1.22 (0.71 ,1.73)
CML333	1.19	20	defg	2.4	20	abcdefg	1.2 (0.8 ,1.61)
Mo17	1.53	17	ab	2.7	20	abc	1.17 (0.7 ,1.64)
CML103	1.37	19	bcde	2.52	20	abcdef	1.15 (0.87 ,1.43)
CML52	0.81	19	klm	1.9	19	hij	1.1 (0.7 ,1.49)
Ki11	1.02	19	fghijkl	2.1	20	efghij	1.08 (0.75 ,1.42)
IL14H	0.96	14	fghijklm	2.03	15	fghij	1.07 (0.8 ,1.34)
B73	0.78	16	lm	1.82	17	ij	1.05 (0.78 ,1.31)
CML277	0.93	20	hijklm	1.96	20	ghij	1.03 (0.6 ,1.45)
CML228	1.38	19	bcd	2.35	20	bcdefgh	0.97 (0.66 ,1.27)
M37W	0.94	20	hijklm	1.91	19	hij	0.97 (0.38 ,1.57)
CML247	1.08	18	fghij	1.98	20	ghij	0.9 (0.62 ,1.18)
Tx303	0.7	18	mn	1.61	17	j	0.9 (0.46 ,1.34)
Ki3	0.94	20	hijklm	1.79	20	ij	0.85 (0.53 ,1.16)
Ky21	1.23	18	cdef	2.08	18	fghij	0.84 (0.49 ,1.19)
NC358	1.22	17	cdef	2.03	18	fghij	0.8 (0.42 ,1.19)
W22	1.15	17	defgh	1.93	16	ghij	0.78 (0.43 ,1.13)
Oh7b	1.05	20	fghijk	1.81	20	ij	0.76 (0.39 ,1.13)
Tzi8	1.48	15	abc	2.24	19	cdefghi	0.76 (0.38 ,1.15)

Mo18W	0.94	18	hijklm	1.66	20	j	0.72 (0.44, 1)

Table 2 Characterization of six maize lines grown with or without inoculation with *Rhizophagus irregularis* and at three P levels. Shoot dry weight (SDW) and shoot P content (SP) of non-colonized (NC) and mycorrhizal (M*)* plants; Phosphorus obtained from the hyphae compartment (PHC) in M plants; % of root length containing fungal structures in M plants (Col); % of root length containing arbuscules in M plants (Arb); Hyphal length density in the hyphae compartment in M plants (HLD). Numbers are means and standard errors (n=4). Means groups based on least significant difference were calculated separately for each P level. For SDW and SP, means groups were calculated for NC and M plants together. For Col and Arb, means groups were calculated using square root transformed data.

Line	Shoot dry wt (g)		Shoot P content (mg)		P from HC (mg)	Col %	Arb %	HLD (m/g)
	NC	M	NC	M				
				7.9 mg P kg^{-1}				
Oh43	6.3 (1.7) ef	18.6 (1.0) b	9.3 (2.3) ef	39.1 (2.3) a	0.54 (0.06) a	87.8 c	70.4 b	21.4 (2.5) a
Mo18W	4.0 (0.2) f	15.0 (0.5) cd	6.5 (0.4) fg	32.1 (0.2) b	0.34 (0.05) b	94.8 a	79.1 a	13.4 (0.8) bc
Pa36	14.0 (1.6) cd	22.0 (1.9) a	17.3 (0.6) d	39.5 (0.8) a	0.41 (0.06) b	93.9 ab	83.4 a	16.3 (2.9) b
Hp301	3.6 (0.7) f	13.8 (0.6) cd	5.9 (1.2) g	28.3 (1.1) c	0.20 (0.02) c	92.6 abc	77.9 ab	10.5 (0.7) c
B73	7.8 (1.6) e	16.6 (2.1) bc	9.8 (2.3) e	34.2 (1.1) b	0.41 (0.04) b	92.5 abc	84.6 a	17.0 (2.2) ab
Mo17	12.0 (0.5) d	19.0 (1.3) ab	16.7 (0.4) d	33.8 (0.9) b	0.41 (0.02) b	88.0 bc	78.7 ab	13.0 (0.8) bc
				15.5 mg P kg^{-1}				
Oh43	18.3 (1.0) def	23.2 (1.3) b	29.7 (2.1) de	50.7 (1.4) a	0.83 (0.14) ab	89.5 bc	65.5 c	19.4 (3.1) a
Mo18W	12.7 (0.6) g	16.2 (0.9) ef	22.2 (1.0) f	42.3 (1.9) bc	0.68 (0.07) bc	96.8 a	82.1 a	14.7 (1.3) bc
Pa36	18.9 (2.7) cde	22.1 (1.6) bc	30.5 (2.4) d	49.9 (4.4) a	0.72 (0.1) abc	96.6 a	78.5 ab	14.2 (0.7) c
Hp301	15 (0.8) fg	17.5 (0.7) def	25.9 (0.6) def	38.1 (2.1) c	0.54 (0.1) c	91.7 b	76.8 ab	13.2 (1.5) c
B73	19.9 (1.8) bcd	27.1 (1.4) a	27.7 (1.0) de	45.9 (2.2) ab	0.92 (0.03) a	93.0 ab	72.9 b	18.9 (2.2) ab
Mo17	19.3 (1.4) cde	21.8 (0.7) bc	25.1 (2.2) ef	41.4 (0.6) bc	0.76 (0.1) ab	87.9 c	71.8 c	15.0 (0.8) abc
				53.2 mg P kg^{-1}				
Oh43	30.8 (1.9) ab	27.1 (1.4) bc	73.5 (2.5) bc	68.8 (1.3) bcd	0.69 (0.1) b	74.7 b	45.0 a	13.9 (1.3) ab

Mo18W	25.2 (1.3) cd	22.4 (1.3) de	63.0 (3.3) de	58.8 (1.6) e	0.69 (0.03) b	84.0 a	55.7 a	10.2 (0.8) bc
Pa36	30.0 (1.8) ab	31.1 (2.5) a	93.0 (5.2) a	68.9 (2.4) bcd	0.81 (0.2) b	87.7 a	54.1 a	10.5 (1.4) abc
Hp301	27.9 (1.0) abc	21.3 (1.6) e	67.4 (2.6) cd	50.4 (4.3) f	0.53 (0.3) b	69.2 b	43.3 a	7.8 (0.88) c
B73	30.26 (1.14) ab	31.45 (2.19) a	75.66 (5.22) b	67.1 (1.61) cd	1.21 (0.05) a	86.59 a	43.46 a	14.34 (2.4) a
Mo17	24.6 (0.8) cde	26.0 (0.9) cd	63.0 (1.4) de	66.1 (3.0) cde	0.6 (0.2) b	87.1 a	53.7 a	10.6 (2.1) abc

FIGURE LEGENDS

Fig. 1. Maize lines varied in response to inoculation with *Funneliformis mosseae*. (a) Shoot dry weight (SDW, g; normalized with respect to differences among experimental plantings) of 30 diverse maize lines grown for 8 weeks with (M; right box) or without (NC left box) inoculation with the fungus *F. mosseae*. Boxes show 1st quartile, median and 3rd quartile. Whiskers extend to the most extreme points within 1.5x box length; outlying values beyond this range are not shown. The overall mean values of NC (1.05g, n=540) and M (2.16g, n=552) groups are shown by vertical lines. Lines are ordered by increasing mycorrhizal response (M-NC) from top to bottom. Letters adjacent to boxes indicate means groups, calculated for NC and M treatments collectively on the basis of least significant difference at $\alpha = 0.05$. Box shading indicates mean phosphorus content (P^{31}, ppm) in the shoot as determined by ionomic analysis, colour-key shown at the top of the panel. (b) Reaction norms (plot of phenotype against environment) for 30 diverse maize lines, contrasting shoot dry-weight (R SDW, g; residual SDW with respect to overall NC or M mean) of non-inoculated plants (NC) and plants inoculated with *F. mosseae* (M). Segments corresponding to six lines selected for further study are labeled and shown in bold. Point shading indicates shoot P content (ppm) as (a). (c) Shoot dry weight (SDW, g) as a function of shoot P content (P^{31}, ppm) in 30 maize lines inoculated with *F. mosseae* (points correspond to mean values). Linear fit (yellow line) and associated 95% confidence interval (shaded area) shown.

Fig. 2. Plant growth was correlated with phosphorus content in six maize lines inoculated with *Rhizophagus irregularis*. Shoot dry weight (Shoot DW; g) as a function of total phosphorus (P) content (Shoot P; mg) in six maize genotypes grown with (yellow circles) or without (open circles) inoculation

with *R. irregularis*, under three levels of P availability (7.9, 15.5, 53.2 mgP/kg). Points indicate the mean; whiskers extend +/- 1 standard error; trend lines based on a linear fit to individual observations for each treatment.

Fig. 3. Phosphorus uptake from the hyphal compartment was correlated with the abundance of root-external hyphae in six maize lines inoculated with *Rhizophagus irregularis*. (a) Percentage of total root length containing mycorrhizal structures (brown) and arbuscules (yellow) in six maize lines, grown under three levels of phosphorus (P) availability (7.9, 15.5 and 53.2 mgP Kg^{-1}) and inoculated with *R. irregularis*. Boxes show 1st quartile, median and 3rd quartile. Whiskers extend to the most extreme points within 1.5x box length. (b) Shoot P acquired from the hyphal compartment (Shoot P from ^{33}P soil, mg) as a function of the length of root-external hyphal (m g^{-1} soil).

Fig. 4. Accumulation of *ZmPt* transcripts responded in inoculation with *Rhizophagus irregularis*. Accumulation of six *ZmPt* (*Pt*) transcripts in root-samples of the lines B73, Mo17, Hp301, Pa36, Mo18W and Oh43, grown under low phosphorus (P) availability (7.9 mg kg^{-1}) with (M) or without (NC) inoculation with *R. irregularis*. Mean absolute transcript accumulation was determined from three biological replicates and scaled independently for each gene panel from white (minimum) to brown (maximum) accumulation. Letters indicate means groups based on least significant difference at $\alpha = 0.05$, calculated independently for each gene. Accumulation of the maize marker transcript *ZmAm3* and the fungal transcript *RiEF* also shown.

Fig. 5. Molecular and physiological patterns were correlated in maize plants inoculated with *Rhizophagus irregularis.* Principle component analysis (PCA) of plant-growth, physiological and molecular observations of six maize lines grown under low phosphorus (P) availability (7.9 mg kg^{-1}) and inoculated with *R. irregularis*. . Biplot showing scores in the first two principal components (PC1: x-axis, PC2: y-axis) for traits (black arrows: shoot dry weight (shoot DW), shoot P, root dry weight (root DW), root P, total colonization (%Col), arbuscule abundance (%Arb), length of root-external hyphae (Hyphae), P uptake from the hyphal compartment (PHC), accumulation of *Pt1*, *Pt3*, *Pt4*, *Pt5*, *Pt6*, *Am3* and *RiEF* transcripts) and lines (B73,

Mo17, HP301, Pa36, Mo18W, Oh43). Points indicating the different lines are coloured by mycorrhizal response (MR, g) calculated as the difference in shoot dry weight in colonized and non-colonized plants.

REFERENCES

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* **215**: 403-410.

Angelard C, Colard A, Niculita-Hirzel H, Croll D, Sanders IR. 2010. Segregation in a mycorrhizal fungus alters rice growth and symbiosis-specific gene transcription. *Curr Biol* **20**: 1216-21.

Baxter IR, Vitek O, Lahner B, Muthukumar B, Borghi M, Morrissey J, Guerinot M Lou, Salt DE. 2008. The leaf ionome as a multivariable system to detect a plant's physiological status. *Proc Natl Acad Sci USA* **105**: 12081–6.

Baxter IR. 2015. Should we treat the ionome as a combination of individual elements, or should we be deriving novel combined traits? J Exp Bot **66**: 2127–31.

Bucher M. 2007. Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. *New Phytol* **173**: 11–26.

Bun-Ya M, Nishimura M, Harashima S, Oshima Y. 1991. The PHO84 gene of *Saccharomyces cerevisiae* encodes an inorganic phosphate transporter. *Mol Cell Biol* **11**: 3229–38.

Breuillin-Sessoms F, Floss DS, Gomez SK, Pumplin N, Ding Y, Levesque-Tremblay V, Noar RD, Daniels DA, Bravo A, Eaglesham JB, et al. 2015. Suppression of arbuscule degeneration in *Medicago truncatula phosphate transporter4* mutants is dependent on the Ammonium Transporter 2 family protein AMT2;3. *Plant Cell* **27**: 1352–66.

Ceasar SA, Hodge A, Baker A, Baldwin SA. 2014. Phosphate concentration and arbuscular mycorrhizal colonisation influence the growth, yield and expression of twelve *PHT1* family phosphate transporters in foxtail millet (*Setaria italica*). *PLoS ONE* **9**: e108459.

Chu Q, Wang X, Yang Y, Chen F, Zhang F, Feng G. 2013. Mycorrhizal responsiveness of maize (*Zea mays* L.) genotypes as related to releasing date and available P content in soil. *Mycorrhiza* **23**: 497–505.

Davidson RM, Hansey CN, Gowda M, Childs KL, Lin H, Vaillancourt B, Sekhon RS, De Leon N, Kaeppler SM, Jiang N, Buell CR. 2011. Utility of RNA sequencing for analysis of maize reproductive transcriptomes. *Plant Genome* **4**: 191–203.

Dray S, Dufour, AB (2007). The ade4 package: implementing the duality diagram for ecologists. Journal of Statistical Software. 22(4): 1-20.

Edgar, RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**, 1792-1797.

Fester T, Sawers R. 2011. Progress and Challenges in Agricultural Applications of Arbuscular Mycorrhizal Fungi. *Crit Rev Plant Sci* **30**: 459–470.

Gerlach N, Schmitz J, Polatajko A, Schlüter U, Fahnenstich H, Witt S, Fernie AR, Uroic K, Scholz U, Sonnewald U *et al*. 2015. An integrated functional approach to dissect systemic responses in maize to arbuscular mycorrhizal symbiosis. *Plant Cell Environ* **38**: 1591–612.

Glassop D, Smith SE, Smith FW. 2005. Cereal phosphate transporters associated with the mycorrhizal pathway of phosphate uptake into roots. *Planta* **222**: 688–698.

Goodstein, DM, Shu, S, Howson, R, Neupane, R, Hayes, RD, Fazo, J, Mitros, T, Dirks, W, Hellsten, U, Putnam, N, and Rokhsar, DS. 2012. Phytozome: a comparative platform for green plant genomics. *Nucleic Acids Res* **40**, D1178-1186.

Grace EJ, Cotsaftis O, Tester M, Smith FA, Smith SE. 2009. Arbuscular mycorrhizal inhibition of growth in barley cannot be attributed to extent of colonization, fungal phosphorus uptake or effects on expression of plant phosphate transporter genes. *New Phytol* **181**: 938–94.

Guimil S, Chang HS, Zhu T, Sesma A, Osbourn A, Roux C, Ioannidis V, Oakeley EJ, Docquier M, Descombes P, Briggs SP *et al.* 2005. Comparative transcriptomics of rice reveals an ancient pattern of response to microbial colonization. *Proc Natl Acad Sci U S A* **102**: 8066–8070.

Gutjahr C, Banba M, Croset V, An K, Miyao A, An G, Hirochika H, Imaizumi-Anraku H, Paszkowski U. 2008. Arbuscular mycorrhiza-specific signaling in rice transcends the common symbiosis signaling pathway. *Plant Cell* **20**: 2989–3005.

Gutjahr C, Sawers RJH, Marti G, Andrés-Hernández L, Yang SY, Casieri L, Angliker H, Oakeley EJ, Wolfender JL, Abreu-Goodger C, Paszkowski U. 2015. Transcriptome diversity among rice root types during asymbiosis and interaction with arbuscular mycorrhizal fungi. *Proc Natl Acad Sci U S A.* **112**: 6754-9.

Harrell FE (2016). Hmisc: Harrell Miscellaneous. R package version 3.17-4. https://CRAN.R-project.org/package=Hmisc

Harrison MJ, Dewbre GR, Liu J. 2002. A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. *Plant Cell* **14**: 2413–2429.

Hetrick BAD, Wilson GWT, Cox TS. 1992. Mycorrhizal dependence of modern wheat varieties, landraces, and ancestors. *Can J Bot* **70**: 2032–2040.

Hetrick BAD, Wilson GWT, Cox TS. 1996. Mycorrhizal response in wheat cultivars: relationship to phosphorus. *Can J Bot* **74**: 19–25.

Hong JJ, Park Y-S, Bravo A, Bhattarai KK, Daniels DA, Harrison MJ. 2012. Diversity of morphology and function in arbuscular mycorrhizal symbioses in *Brachypodium distachyon*. *Planta* **236**: 851–65.

Jakobsen I, Abbott LK, Robson AD. 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L.. *New Phytol* **120**: 371-380.

Jakobsen I, Gazey C, Abbott LK. 2001. Phosphate transport by communities of arbuscular mycorrhizal fungi in intact soil cores. *New Phytol* **149**: 95–103.

Janos DP. 2007. Plant responsiveness to mycorrhizas differs from dependence upon mycorrhizas. *Mycorrhiza* **17**: 75–91.

Javot H, Penmetsa R V, Terzaghi N, Cook DR, Harrison MJ. 2007. A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. *Proc Natl Acad Sci USA* **104**: 1720–1725.

Kaeppler SM, Parke JL, Mueller SM, Senior L, Stuber C, Tracy WF. 2000. Variation among maize inbred lines and detection of quantitative trait loci for growth at low phosphorous and responsiveness to arbuscular mycorrhizal fungi. *Crop Sci* **40**: 358–364.

Karandashov V, Bucher M. 2005. Symbiotic phosphate transport in arbuscular mycorrhizas. *Trends Plant Sci* **10**: 22–9.

Kobae Y, Hata S. 2010. Dynamics of periarbuscular membranes visualized with a fluorescent phosphate transporter in arbuscular mycorrhizal roots of rice. *Plant Cell Physiol* **51**: 341–53.

Koide RT, Li M, Lewis J, Irby C. 1988. Role of mycorrhizal infection in the growth and reproduction of wild vs. cultivated plants. *Oecologia* **77**: 537–543.

Kormanik PP, McGraw A-C. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. In: Schenck NC, ed. *Methods and Principles of Mycorrhizal Research.* St Paul, Minnesota: American Phytopathol Soc., 37-46.

Li P, Ponnala L, Gandotra N, Wang L, Si Y, Tausta SL, Kebrom TH, Provart N, Patel R, Myers CR, *et al.* 2010. The developmental dynamics of the maize leaf transcriptome. *Nat Genet* **42**: 1060–1067.

Liu F, Xu Y, Jiang H, Jiang C, Du Y, Gong C, Wang W, Zhu S, Han G, Cheng B. 2016. Systematic identification, evolution and expression analysis of the *Zea mays PHT1* gene family reveals several new members involved in root colonization by arbuscular mycorrhizal fungi. *Int J Mol Sci* **17**: 10.3390/ijms17060930

Liu Y, Shi G, Mao L, Cheng G, Jiang S, Ma X, An L, Du G, Collins Johnson N, Feng H. 2012. Direct and indirect influences of 8 yr of nitrogen and phosphorus fertilization on *Glomeromycota* in an alpine meadow ecosystem. *New Phytol* **194**: 523–535.

Lynch JP. 2011. Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. *Plant Physiol* **156**: 1041–1049.

Maeda D, Ashida K, Iguchi K, Chechetka SA, Hijikata A, Okusako Y, Deguchi Y, Izui K, Hata S. 2006. Knockdown of an arbuscular mycorrhiza-inducible phosphate transporter gene of *Lotus japonicus* suppresses mutualistic symbiosis. *Plant Cell Physiol* **47**: 807–17.

de Mendiburu F. 2016. agricolae: Statistical Procedures for Agricultural Research. R package version 1.2-4. https://CRAN.R-project.org/package=agricolae

McMullen MD, Kresovich S, Villeda HS, Bradbury P, Li H, Sun Q, Flint-Garcia S, Thornsberry J, Acharya C, Bottoms C, *et al.* 2009. Genetic properties of the maize nested association mapping population. *Science* **325**: 737–40.

Mudge SR, Rae AL, Diatloff E, Smith FW. 2002. Expression analysis suggests novel roles for members of the *Pht1* family of phosphate transporters in *Arabidopsis*. *Plant J* **31**: 341-353.

Munkvold L, Kjøller R, Vestberg M, Rosendahl S, Jakobsen I. 2004. High functional diversity within species of arbuscular mycorrhizal fungi. *New Phytol* **164**: 357–364.

Nagy R, Karandashov V, Chague V, Kalinkevich K, Tamasloukht M, Xu G, Jakobsen I, Levy AA, Amrhein N, Bucher M. 2005. The characterization of novel mycorrhiza-specific phosphate transporters from *Lycopersicon esculentum* and *Solanum tuberosum* uncovers functional redundancy in symbiotic phosphate transport in solanaceous species. *Plant J* **42**: 236–50.

Nazeri NK, Lambers H, Tibbett M, Ryan MH. 2013. Do arbuscular mycorrhizas or heterotrophic soil microbes contribute toward plant acquisition of a pulse of mineral phosphate? *Plant Soil* **373**: 699–710.

Nagy R, Vasconcelos MJ V, Zhao S, McElver J, Bruce W, Amrhein N, Raghothama KG, Bucher M. 2006. Differential regulation of five *Pht1* phosphate transporters from maize (*Zea mays* L.). *Plant Biol* **8**: 186–197.

Newman EI. 1966. A method for estimating the total length of root in a sample. *J Appl Ecol* **3**: 139-145.

Olsen SR, Cole CV, Watanabe FS, Dean LA. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *USDA Circular* No 939.

Parniske M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nat Rev Microbiol* **6**: 763–775.

Paszkowski, U, Boller, T. 2002. The growth defect of *lrt1*, a maize mutant lacking lateral roots, can be complemented by symbiotic fungi or high phosphate nutrition. *Planta* **214**: 584–590.

Paszkowski U, Kroken S, Roux C, Briggs SP. 2002. Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. *Proc Natl Acad Sci USA* **99**: 13324–13329.

Paszkowski U, Jakovleva L, Boller T. 2006. Maize mutants affected at distinct stages of the arbuscular mycorrhizal symbiosis. *Plant J* **47**: 165–173.

Pearson JN, Jakobsen I. 1993. The relative contribution of hyphae and roots to phosphorus uptake by arbuscular mycorrhizal plants measured by dual labelling with ^{32}P and ^{33}P. *New Phytol* **124**: 489–494.

Perez-Montano F, Alias-Villegas C, Bellogin RA, del Cerro P, Espuny MR, Jimenez-Guerrero I, Lopez-Baena FJ, Ollero FJ, Cubo T. 2014. Plant growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production. *Microbiol Res* **169**: 325–336.

Pumplin N, Zhang X, Noar RD, Harrison MJ. 2012. Polar localization of a symbiosis-specific phosphate transporter is mediated by a transient reorientation of secretion. *Proc Natl Acad Sci USA* **109**: E665-672.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rausch C, Daram P, Brunner S, Jansa J, Laloi M, Leggewie G, Amrhein N, Bucher M. 2001. A phosphate transporter expressed in arbuscule-containing cells in potato. *Nature* **414**: 462–70.

Rose TJ, Rose MT, Pariasca-Tanaka J, Heuer S, Wissuwa M. 2011. The Frustration with Utilization: Why Have Improvements in Internal Phosphorus Utilization Efficiency in Crops Remained so Elusive? *Front Plant Sci* **2**: 73.

Sawers RJH, Gebreselassie MN, Janos DP, Paszkowski U. 2010. Characterizing variation in mycorrhiza effect among diverse plant varieties. *Theor Appl Genet* **120**: 1029–1039.

Sawers RJ, Gutjahr C, Paszkowski U. 2008. Cereal mycorrhiza: an ancient symbiosis in modern agriculture. *Trends Plant Sci* **13**: 93–97.

Schnepf A, Roose T, Schweiger P. 2008. Impact of growth and uptake patterns of arbuscular mycorrhizal fungi on plant phosphorus uptake - A modelling study. *Plant Soil* **312**: 85–99.

Schweiger PF, Jakobsen I. 1999. Direct Measurement of Arbuscular Mycorrhizal Phosphorus Uptake into Field-Grown Winter Wheat. *Agronomy J* **91**: 998-1002.

Schweiger PF, Thingstrup I, Jakobsen I. 1999. Comparison of two test systems for measuring plant phosphorus uptake via arbuscular mycorrhizal fungi. *Mycorrhiza* **8**: 207–213.

Sen, TZ, Harper, LC, Schaeffer, ML, Andorf, CM, Seigfried, T, Campbell, DA, Lawrence, CJ. 2010. Choosing a genome browser for a model organism database: surveying the maize community. *Database*: baq007.

Schnable JC, Springer NM, Freeling M. 2011. Differentiation of the maize subgenomes by genome dominance and both ancient and ongoing gene loss. *Proc Natl Acad Sci USA* **108**: 4069–4074.

Schnable, PS, Ware D, Fulton RS, Stein JC, Wei F, Pasternak S, Liang C, Zhang J, Fulton L, Graves TA, et al. 2009. The B73 maize genome: complexity, diversity, and dynamics. *Science* **326**: 1112-1115.

Smith SE, Read DJ. 2008. *Mycorrhizal symbiosis*. Cambridge, UK: Academic Press.

Smith SE, Smith FA, Jakobsen I. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiol* **133**: 16–20.

Smith SE, Smith FA, Jakobsen I. 2004. Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. *New Phytol* **162**: 511-524.

Sokolski S, Sguin S, Khasa D, Levesque CA, Piche Y. 2010. Conspecificity of DAOM 197198, the model arbuscular mycorrhizal fungus, with *Glomus irregulare*: molecular evidence with three protein-encoding genes. *Botany* **88**: 829-838.

Syers J, Johnston A, Curtin D. 2008. Efficiency of soil and fertilizer phosphorus use Reconciling changing concepts of soil phosphorus behaviour with agronomic information. *FAO Fertilizer and Plant Nutrition Bulletin no 18.* Rome, Italy: FAO

Vance CP. 2014. Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. *Plant Physiol* **127**: 390–397.

Veneklaas EJ, Lambers H, Bragg J, Finnegan PM, Lovelock CE, Plaxton WC, Price CA, Scheible WR, Shane MW, White PJ, Raven J. 2012. Opportunities for improving phosphorus-use efficiency in crop plants. *New Phytol* **195**: 306–320.

Volpe V, Giovannetti M, Sun XG, Fiorilli V, Bonfante P. 2016. The phosphate transporters *LjPT4* and *MtPT4* mediate early root responses to phosphate status in non mycorrhizal roots. *Plant Cell Environ* **39**: 660-71.

Walder F, Brulé D, Koegel S, Wiemken A, Boller T, Courty P-E. 2015. Plant phosphorus acquisition in a common mycorrhizal network: regulation of phosphate transporter genes of the *Pht1* family in sorghum and flax. *New Phytol* **205**: 1632–45.

Walder F, Niemann H, Natarajan M, Lehmann MF, Boller T, Wiemken A. 2012. Mycorrhizal networks: common goods of plants shared under unequal terms of trade. *Plant Physiol* **159**: 789–97.

Wei T, Simko V. 2016. corrplot: Visualization of a Correlation Matrix. R package version 0.77. https://CRAN.R-project.org/package=corrplot

Willmann M, Gerlach N, Buer B, Polatajko A, Nagy R, Koebke E, Jansa J, Flisch R, Bucher M. 2013. Mycorrhizal phosphate uptake pathway in maize: vital for growth and cob development on nutrient poor agricultural and greenhouse soils. *Front Plant Sci* **4**: 533.

Wang X, Elling AA, Li X, Li N, Peng Z, He G, Sun H, Qi Y, Liu XS, Deng XW. 2009. Genome-wide and organ-specific landscapes of epigenetic modifications and their relationships to mRNA and small RNA transcriptomes in maize. *Plant Cell* **21**: 1053–1069.

Yang SY, Grønlund M, Jakobsen I, Grotemeyer MS, Rentsch D, Miyao A, Hirochika H, Kumar CS, Sundaresan V, Salamin N *et al.* 2012. Nonredundant regulation of rice arbuscular mycorrhizal symbiosis by two members of the *PHOSPHATE TRANSPORTER1* gene family. *Plant Cell* **24**: 4236–4251.

Yao Q, Li XL, Feng G, Christie P. 2001. Influence of extramatrical hyphae on mycorrhizal dependency of wheat genotypes. *Commun Soil Sci Plant Anal* **32**: 3307–3317.

Ziegler G, Terauchi A, Becker A, Armstrong P, Hudson K, Baxter I. 2013. Ionomic screening of field-grown soybean identifies mutants with altered seed elemental composition. *Plant Genome* **6**: 1–9.

SUPPORTING INFORMATION

The following Supporting Information is available for this article:

Fig. S1 Inoculation with *Funneliformis mosseae* promotes growth in a panel of 30 diverse maize lines under low phosphorus availability

Fig. S2 PHT1 protein tree

Fig. S3 Maize *PHT1* transcripts accumulate throughout the plant life-cycle

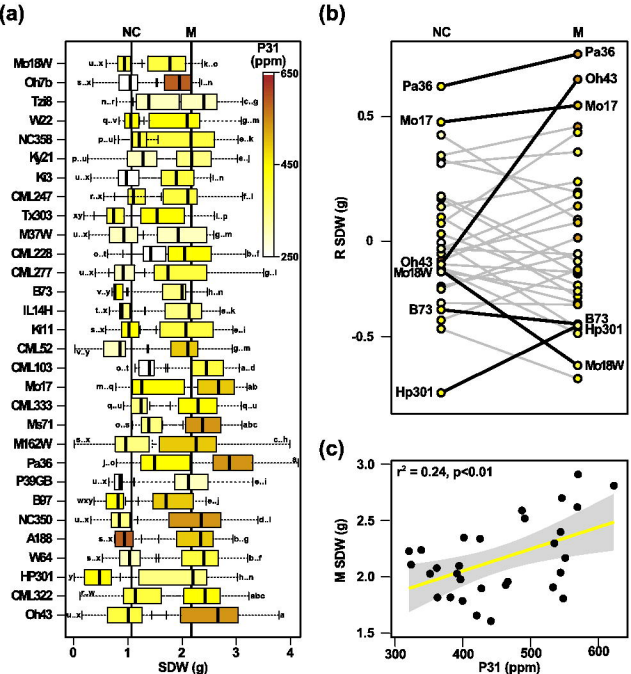
Fig. S4 Accumulation of transcripts encoding PHT1 proteins responds to phosphorus availability and inoculation with *Rhizophagus irregularis*.

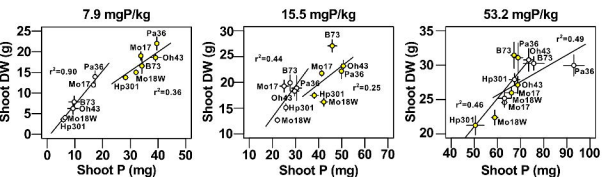
Fig. S5 Accumulation of transcripts encoding PHT1 proteins is correlated with phosphorus accumulation and inoculation with *Rhizophagus irregularis* across diverse maize lines.

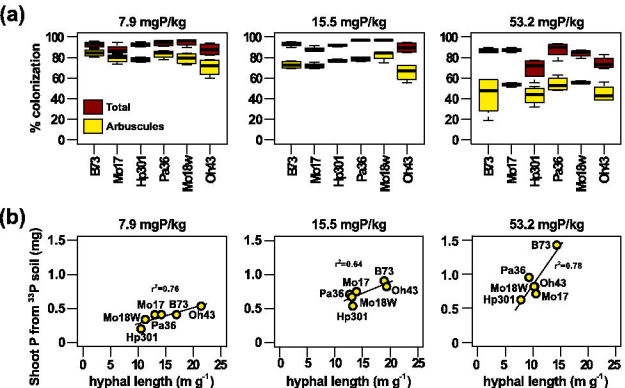
Table S1 Phosphorus concentration in the leaves of 30 maize lines grown with or without inoculation with *Funneliformis mosseae.*

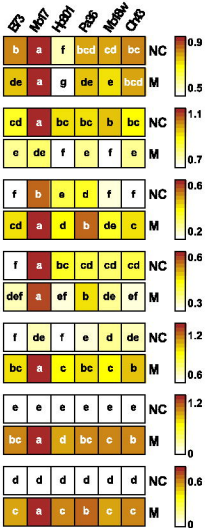
Table S2 Primers used for real time PCR

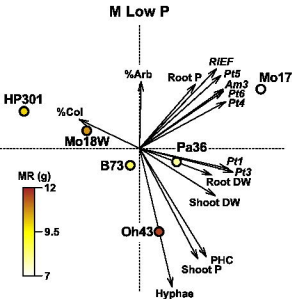
396


(a)
NC
M
P31 (ppm)
650
450
250
Mo18W
Oh7b
Tzi8
W22
NC358
Ky21
K63
CML247
Tx303
M37W
CML228
CML277
B73
IL14H
K11
CML52
CML103
Mo17
CML333
Ms71
M162W
Pa36
P39GB
B97
NC350
A188
W64
HP301
CML322
Oh43
0
1
2
3
4
SDW (g)
(b)
NC
M
Pa36
Oh43
Mo17
Oh43
Mo18W
B73
Hp301
Pa36
Mo17
B73
Hp301
Mo18W
0.5
0
-0.5
R SDW (g)
(c)
r² = 0.24, p<0.01
3.0
2.5
2.0
1.5
M SDW (g)
300
400
500
600
P31 (ppm)


7.9 mgP/kg
Shoot DW (g)
Shoot P (mg)
r²=0.90
r²=0.36
Pa36
Mo17
Oh43
B73
Mo18W
Hp301
15.5 mgP/kg
r²=0.44
r²=0.25
53.2 mgP/kg
r²=0.49
r²=0.46


(a)
7.9 mgP/kg
15.5 mgP/kg
53.2 mgP/kg
% colonization
Total
Arbuscules
B73
Mo17
Hp301
Pa36
Mo18w
Oh43
(b)
Shoot P from 33P soil (mg)
hyphal length (m g-1)
r2=0.76
r2=0.64
r2=0.78


EF3
Mol7
Hsp11
Pas6
Mo6w
Chr6
Pt1
b a f bcd cd bc NC
de a g de e bcd M
0.9
0.5
Pt3
cd a bc bc b bc NC
e de f e f e M
1.1
0.7
Pt4
f b e d f f NC
cd a d b de c M
0.6
0.2
Pt5
f a bc cd cd cd NC
def a ef b de ef M
0.6
0.3
Pt6
f de f e d de NC
bc a c bc c b M
1.2
0.6
AM3
e e e e e e NC
bc a d bc c b M
1.2
0
RiEF
d d d d d d NC
c a c b c c M
0.6
0


M Low P
%Arb
RIEF
Root P
Pt5
Am3
Pt6
Pt4
Mo17
HP301
%Col
Mo18W
Pa36
B73
Pt1
Pt3
Root DW
Shoot DW
MR (g)
12
9.5
7
Oh43
PHC
Shoot P
Hyphae