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

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SUMMARY

- Plant interactions with arbuscular mycorrhizal fungi have long excited interest for their potential to promote more efficient use of mineral resources in agricultural production. Their use, however, remains limited, in part because of a lack of understanding of the factors that determine symbiotic outcome. In this work, variation in response to arbuscular mycorrhizal colonization was characterized a panel of genetically diverse maize inbred lines.
- The parents of the maize Nested Association Mapping population were evaluated, with and without colonization, in early vegetative stages. Subsequently, six lines with contrasting phenotypes were selected for further characterization, including quantification of fungal colonization, mycorrhiza-mediated phosphorus uptake, and accumulation of transcripts encoding plant PHT1 family phosphate transporters.
- The relative growth of lines changed between non-inoculated and inoculated plants, indicative of variation in host capacity to profit from symbiosis. Patterns of *Pht1* transcript accumulation varied among lines, and were correlated with outcome.
- Larger growth responses were correlated with more extensive development of root-external hyphae, increased accumulation of specific *Pht1* transcripts and a high level of mycorrhiza-mediated phosphorus uptake. The data suggest that host genetic factors influence fungal growth strategy with subsequent impact on plant biomass production.

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

INTRODUCTION

The rising cost of agricultural inputs and an increasing awareness of the negative environmental consequences have resultes in ever greater interest in beneficial crop-microbe interactions and their potential 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).

AM fungi provide the plant host with greater access to soil nutrients and water through connection to a network of fungal hyphae more extensive than the plant's own root system (Bucher, 2007). In addition, AM symbioses have been implicated in enhanced tolerance to a range of abiotic and biotic stresses (Smith & Read, 2008). Such benefits are not provided without cost, however, and the plant host must provide carbohydrates to the fungus, which represents a diversion of photosynthetically fixed carbon away from primary productivity and yield. Ultimately, the outcome, which may be positive or negative, is dependent 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). Indeed, in high-input modern agricultural systems, the benefit of the symbiosis to the plant may be

marginal (Grace *et al.*, 2009), and it has been hypothesized that conventional breeding practices may have promoted weakening of the mutualism (Hetrick *et al.*, 1992, 1996). Comparisons of mycorrhizal response, however, can be complicated by variation in overall plant adaptation to a given set of conditions - poorly adapted plants will typically show the greatest performance increase following AM colonization (e.g. Hetrick *et al*., 1992; Kaeppler *et al*., 2000), although such improvement need not indicate a superior capacity to benefit from colonization *per se* (Sawers *et al.*, 2010). The maize mutant *lrt1,* which exhibits reduced lateral root development, illustrates an extreme case: high dependency under low P availability that can be largely compensated by the formation of AM symbiosis (Paszkowski & Boller, 2002). The question remains as to whether , and how, certain varieties derive greater benefit from AM symbioses than others and to what extent plant breeding can optimize these interactions for agricultural systems (Sawers *et al.*, 2008; Fester & Sawers, 2011). A better understanding of the molecular and physiological impact of AM symbiosis has the potential to enhance greatly interpretation of outcome variation.

The best characterized benefit of AM symbiosis is enhanced plant phosphorus (P) nutrition. Given that limited P availability is a major check on global agricultural production and food security, assessment of AM outcome in terms of P nutrition is a justifiable approximation of this complex symbiotic trade-off. 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 at al., 2011; Veneklaas *et al.*, 2012), AM symbiosis most directly impacting the former. Levels of P fertilizer uptake in agricultural systems are typically low (15-20%; Syers *et al.*, 2008), largely as a result of the relative immobility of P in the soil and the ready

formation of a zone of P depletion around the root (Bucher, 2007). Optimization of the root system architecture can contribute significantly to P foraging (Lynch, 2011), but, under a given set of conditions, AM symbioses may present the greatest opportunity to access a greater soil volume. Physiological studies have demonstrated that symbiotic phosphate uptake 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 phosphate taken up by a plant may be acquired via the symbiotic route (Schweiger and Jakobsen, 1999; Smith *et al.*, 2003; Yang *et al.*, 2012).

Molecular analyses have supported the distinction between symbiotic and direct phosphate uptake, identifying the various members of the plant PHT1 P transporter family to play roles specific to the the two pathways. Where plants are competent to host AMF, there is at least one family member (PT11 in rice and variously named orthologs in other species) acting predominantly, or exclusively, during AM symbiosis (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). Mutant analysis has demonstrated PT11 proteins to be essential for formation and maintenance of AM symbiosis (Maeda *et al.*, 2006; Javot *et al.*, 2007; Yang *et al.*, 2012), although this phenotype has been partially rescued by nitrogen starvation in medic (Breuillin-Sessoms *et al.*, 2015) and co-cultivation with wild-type plants in maize (Willmann *et al.*, 2013). In medic, the PT11 protein MtPT4 has been localized to the peri-arbuscular membrane (Harrison *et al.*, 2002; Kobae & Hata, 2010; Pumplin *et al.*, 2012), and the PT11 proteins are considered to provide the principal route of P uptake from fungus to plant.

In this study, differences in mycorrhiza response among a panel of diverse maize lines are dissected to identify variation linked to a greater ability of the host to profit from the symbiosis. Selected lines were further characterized by quantification of AM-mediated and total P uptake, fungal colonization of roots and soil, and accumulation of transcripts encoding PHT1 family P transporters.

MATERIALS AND METHODS

Evaluation of response to AMF 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 lines B73, W22 and W64A (a line used previously for study of AM symbiosis; Paszkowski *et al.*, 2006), was evaluated in one litre pots, under conditions of low phosphorus availability, with or without inoculation with *Funneliformis mosseae* (isolate number 12, European Bank of Glomales, http://www.kent.ac.uk/bio/beg/), as previously described (Sawers *et al.*, 2010). At 8 weeks after emergence, the aerial part of the plant was harvested, dried and weighed. Six experiments (A-F) were conducted in the greenhouse facility at the University of Lausanne, Switzerland, during the period 2007 - 2010. Each treatment was replicated three times in each experiment, with the exception of experiment D in which each treatment was replicated five times. Shoot dry weight data was analyzed without further transformation for clarity. Systematic variation among experiments was eliminated using linear estimation. The experiment effect was estimated separately for non-inoculated and inoculated plants. Mycorrhiza response was estimated for each genotyping by calculation of a *t*-interval for the difference of inoculated and non-inoculated means. All

analysis was performed using R statistics (www.r-project.org). See *Supporting Information* for raw data and further details.

Determination of elemental concentration by ICP-MS analysis

Tissue samples were weighed then 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 in Ziegler *et al.*, 2013. Elemental concentrations of B, Na, Mg, Al, P, S, K, Ca, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Mo, and Cd were measured using an Elan 6000 DRC-e mass spectrometer (Perkin-Elmer SCIEX) connected to a PFA microflow nebulizer (Elemental Scientific) and Apex HF desolvator (Elemental Scientific) using the procedure described in Ziegler *et al.* To correct for machine drift both during a single run and between runs, a control solution is run every tenth sample. All analysis was performed using R statistics. See *Supporting Information* for raw data and full analysis.

Characterization of AM phosphorus uptake in six selected lines

Six maize lines with different low P tolerance were selected and evaluated at the Technical University of Denmark. Plants were grown in 2.4 L PVC tubes in accordance with Smith *et al.*, 2003. The growth medium (hereafter referred to as soil) contained 7.9 mg 0.5M bicarbonate-extractable P kg^{-1} (Olsen *et al.*, 1954) and was a 1:1 (w:w) mixture of sand and irradiated soil (10 kGy, 10 MeV electron beam) that received basal nutrients (Pearson & Jakobsen, 1993) and KH_2PO_4 at nil, 15 or 90 mg P kg^{-1}. All nutrients were added in solution and carefully mixed into the soil. The root plus hyphal compartment (RHC) contained 2750 g soil and the hyphal compartment (HC) was a small plastic vial placed in the middle of the RHC. The HC contained 55 g of ^{33}P labeled soil (5 kBq g^{-1}) and lined with a 25 µm nylon

mesh at both ends to prevent root in-growth. Seven weeks later, bicarbonate extracts had a specific activity (SA = $^{33}P/^{31}P$) of 144.7, 79.9 or 29.4 kBq mg^{-1} P in soil amended with nil, 15 or 90 mg P kg^{-1}. Each maize line was grown in 8 replicate pots in half of which 140 g dry soil-root inoculum of *Rhizophagus irregularis* BEG87 was thoroughly mixed into the growth medium. Filtered BEG87 inoculum leachings were added to all pots as an attempt to establish the same soil microbial community (Pearson & Jakobsen, 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. In addition to the initial basal nutrient dressing, supplemental N (NH_4NO_3), Mg and S ($MgSO_4^{2-}$) was added 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. Roots system was 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 in bicarbonate extracts of HC soil were used to estimate the relative contribution of the AM pathway to total shoot P uptake as described in Smith *et al.* (2004). Root length

was measured by image analysis using the Win-Rhizo software (Win-Rhizo version 2009b, Regent Instruments, Canada) and a scanner (Epson (ModelJ1221A), Seiko Epson Corp. Japan). Images were acquired by placing 1.5 g untangled roots (FWRL), from the RHC subsample in a water filled Plexiglas tray (17.5 x 23.9 cm). Total root length of each plant was calculated as RL x FWRoot x $(FWRL)^{-1}$. 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). Hyphal length was measured by a grid intersection method after wet-sieving of aqueous soil suspensions on membrane filters (Jakobsen *et al.*, 1992). Where appropriate, mycorrhiza response was estimated for each genotyping by calculation of a *t*-interval for the difference of inoculated and non-inoculated means. All analysis was performed using R statistics. See *Supporting Information* for raw data and full analysis

Bioinformatic identification of maize *Pht* genes

To identify a complete set of putative PHT1 encoding genes in maize, the *Saccharomyces cerevisiae* PHO84 protein (Uniprot id P25297) was used as a BlastP query (Altschul *et al.*, 1990) to search the primary transcript predicted protein sequences from version 6a of the annotated B73 maize genome (Schnable *et al.*, 2009), obtained from Phytozome 10 (Goodstein *et al.* 2012). Using a cut-off E-value of $1e^{-54}$, 13 gene-models were retrieved and aligned using MUSCLE (Edgar, 2004). All 13 sequences contained the conserved GGDYPLSATIxSE motif in helix 4 reported previously to be present in PHT proteins (Karandashov & Bucher, 2005). The resulting block-alignment file was converted to Stockholm 1.0 format, and used as input to hmmbuild (HMMER suite version 3.1b2) to

search (hmmsearch) the maize primary transcript predicted protein sequences for additional PHT1 proteins. 35 new protein sequences were identified based on an inclusion threshold of E-value <0.01. None of these additional sequences, however, contained the conserved GGDYPLSATIxSE motif and consequently there were not considered to be authentic PHT1 proteins. The final list of 13 maize PHT1 encoding gene models is presented in Supporting Information.

Analysis of *ZmPt* transcript accumulation

A LightCycler 480 SYBR green I master mix kit (Roche; Mannheim, Germany) was used to prepare samples 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 total 6 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 performed using R statistics. See Supporting Information for full analysis.

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, root dry weight, root P, total colonization, arbuscule abundance, length of root-external hyphae, P uptake from the hyphal compartment, transcript accumulation of *Pt6*, *Pt8b*, *Pt11*, *Pt13a*,

184 *Pt13b*, *Am3* and *RiEF*) using rcorr::rcorr in the R stastistics package and the results visualized
185 using corrplot::corrplot. Principal component analysis (PCA) was performed with
186 ade4::dudi.pca in the R statistics package using centered and scaled data, and the results
187 vizualized with ade4::scatter.
188
189 **RESULTS**
190 **Mycorrhiza response is correlated with accumulation of phosphorus in the leaves**
191 To define physiological and molecular patterns correlated with variation in the outcome of
192 AM symbioses, a panel of thirty diverse maize lines (see Materials and Methods), was
193 evaluated under P limiting conditions in the greenhouse, with (M) or without (NC)
194 inoculation with the fungus *Funneliformis mosseae*. Plants were harvested eight weeks after
195 emergence (V8 stage, before the onset of flowering; Counce *et al.*, 2000) and shoot dry-
196 weight (SDW; g) determined (Table 1). Collectively, the evaluated lines showed a positive
197 outcome when inoculated with AMF (Fig. S1), with a significant ($p < 0.001$) increase in
198 mean SDW from 1.05g in NC plants to 2.16g in M plants, equating to a panel-wide
199 mycorrhiza response (MR = M - NC) of 1.1g ±0.08g (95% interval for difference in means).
200 Roots were harvested from a subset of plants, and the abundance of fungal structures
201 quantified by microscopic inspection. NC plants were confirmed to be free of fungal
202 structures. The greatest SDW in NC plants was seen in Pa36 (1.67g), and the lowest in Hp301
203 (0.44g). The level of colonization in M plants was generally high, with a mean of 57% ±0.7%
204 (95% interval for proportion) of root positions examined containing at least one type of
205 fungal structure (hyphae, arbuscules or vesicles), although a broad range of colonization was
206 observed (5% - 98%). At the level of individual lines, all showed increased SDW following

inoculation (Fig. 1a; Table 1). In the panel evaluation, the most responsive line was Oh43 (MR = 1.85g), and the least responsive was Mo18W (MR = 0.72g). The contrast in outcome between Oh43 and Mo18W was reflected by rank-changing shifts in growth relative to other lines in the panel: similar and typical of the panel in the absence of fungus; different and outlying when inoculated (Fig. 1b). As such, Oh43 and Mo18W show similar dependence but a divergent capacity to profit from AM symbiosis.

Evaluation was conducted under P limiting conditions, suggesting that variation in performance would be driven largely by differences in P accumulation. To evaluate plant nutrition status, the accumulation of P and nineteen other elements was quantified in roots and leaves using inductively coupled plasma-mass spectroscopy (ICP-MS; Baxter *et al.*, 2008). Ten elements (Na, Al, P, S, Mn, Fe, Co, Ni, Zn, Cd) were found to accumulate to different levels between M and NC plants, in either roots and/or leaves ($p<0.05$, adjusted for multiple tests; Table S1). Principal component analysis indicated variation in P content was the most important factor differentiating lines and NC and M plants (Fig. S2). Leaf P content was positively correlated with SDW, notably in AM plants (Table 1; Fig. 1; $p<0.01$, $r^2=0.24$). Leaf P content was similar in Mo18W and Oh43 when non-colonized (393ppm and 292ppm, respectively) but diverged when plants were inoculated (422ppm and 625ppm, respectively), mirroring SDW, and suggesting variation in P uptake efficiency to be driving the difference in MR (Fig. S3). On the basis of this initial screen, Mo18W and Oh43 were selected for more detailed physiological study. HP301 and Pa36 were selected also as they showed the lowest and highest dependence (*i.e.* NC SDW), along with the broadly used lines B73 and Mo17.

Mycorrhizal response is correlated with the abundance of root-external hyphae

As plant growth was correlated with P accumulation in the initial screen, it was decided to characterize in detail direct and AM P uptake across a range of P availability in the selected lines Oh43, Mo18W, Pa36, HP301, B73 and Mo17. Plants were grown in a previously described split-compartment system using ^{33}P uptake and accumulation (Smith *et al.*, 2003). SDW increased with greater P addition, irrespective of AMF inoculation (Table2; Fig. 2). In contrast, average MR across lines ranged from positive to negative with increasing P availability (low P MR = 9.56g; medium P MR = 3.95g, high P MR = -1.57g). The relative MR among lines changed also with P availability. Growth at low P was strongly correlated with seed size (Fig. S4). Root dry weight (RDW) was greater in M than in NC plants at low P, was not significantly affected by colonization at medium P and was reduced in M relative to NC plants at high P (data not shown). As observed in the panel evaluation, SDW was correlated with total shoot P content, both among lines and between NC and M treatments (Fig. 2). At low P, MR (in terms of SDW) was higher in Oh43 than in the other lines, consistent with the results of the panel evaluation. Oh43 remained highly responsive at medium P, but exhibited a negative response at high P (Fig. 2). Although showing high MR at low P, Oh43 did not show a greater total root colonization than the other lines (Fig. 3a). Indeed, the proportion of root length containing arbuscules was marginally lower in Oh43 than other lines (Fig. 3a). In contrast, Oh43 supported a significantly greater development of extra-radical hyphae than the other lines at low P, and the greatest AM P uptake (Fig. 3b). Across all lines, the length of root-external hyphae decreased with increasing P availability, although P uptake from HC soil increased (Fig. 3b). B73 continued to supported abundant extra-radical hyphae even at high P availability with a concomitant high level of AM P uptake.

252

253 **The maize genome encodes multiple AMF-responsive PHT1 phosphate transporters**

254 To investigate further the balance of direct and AM P uptake, transcripts encoding *Pht1*
255 phosphate transporters were quantified. A previous report identifying 13 *Pht1*-encoding genes
256 in the maize genome (Liu *et al*., 2016) was confirmed using a hidden Markov search (Table
257 S3). Maize and rice PT protein sequences were aligned, along with a number of previously
258 characterized mycorrhiza associated sequences from other species, and this alignment used to
259 construct a maximum likelihood tree, identifying gene groups and providing the basis for the
260 nomenclature used below (Fig. S5; maize genes are named by similarity to rice following a
261 nomenclature reported in doi: http://dx.doi.org/10.1101/042028. A key relating the
262 nomenclature used here to that of Liu *et al.* is provided in Table S3.)

263 ZmPT11 (previously reported as ZmPHT1;6. Nagy *et al.*, 2006; Willmann *et al.*, 2013)
264 was the unique maize member of a group including the well-characterized mycorrhiza-
265 associated proteins medic MtPT4 (Harrison *et al.*, 2002), rice OsPT11 (Paszkowski *et al.*,
266 2002), potato StPT4 (Nagy *et al.*, 2005) and tomato LePT4 (Nagy *et al.*, 2005). ZmPT14 was
267 the only maize member of a second mycorrhiza-associated group that included barley HvPT8
268 and wheat TaPT1:myc (Glassop *et al.*, 2005). ZmPT9 and ZmPT13a-d defined a further
269 group with the mycorrhiza-associated rice protein OsPT13 (Yang *et al.*, 2012). The remaining
270 ZmPT proteins belonged to a larger group that contained a number of rice proteins
271 characterized previously to play roles in direct P uptake and translocation.

272 To gain a general panorama, *ZmPt* transcript accumulation was investigated using two
273 existing seedling transcriptome datasets (Wang *et al.*, 2009; Li *et al.*, 2010) and and a third
274 set profiling reproductive tissues (Davidson *et al.*, 2011). Representative *Pt* transcripts were

found in all tissues and developmental stages examined, indicating the importance of the family throughout the plant life cycle, while revealing specialization at the level of transcript accumulation (Fig. S6). Ten *Pt* transcripts were selected and quantified directlt in the roots and shoots of B73 seedlings, grown under low (10µM), moderate (100µM) or high (1000µM) P availability in the absence of AM colonization, or under moderate P with *R. irregularis* inoculation. Seven of ten selected *Pt* transcripts (*Pt6*, *Pt7*, *Pt9*, *Pt11*, *Pt13a*, *Pt13b* and *Pt14*) accumulated differentially between NC and M plants, in at least one of the tissues assayed (Tukey HSD, α=0.05; Fig. 4). The transcripts *Pt7*, *Pt9*, *Pt11*, *Pt13a* and *Pt14* accumulated to significantly higher levels in the roots of M plants compared to NC plants. In the case of *ZmPt14*, transcripts accumulated exclusively in the roots of M plants. Transcripts encoded by *ZmPt11* were the most abundant in colonized roots, although they were present also at lower levels in roots and shoots of NC plants. The transcripts *Pt6*, *Pt9*, *Pt11*, *Pt13a* and *Pt13b* accumulated to significantly lower levels in the leaves of M plants compared to NC plants. With the exception of *ZmPt14*, all transcripts were detected in NC plants in at least one of the tissues assayed.

Accumulation of *ZmPt* transcripts is correlated with mycorrhizal P uptake

To investigate the relationship between AM outcome and *Pt* function, accumulation of *Pt6*, *Pt8b*, *Pt11*, *Pt13a* and *Pt13b* transcripts was quantified in root samples collected from the characterization of six selected lines. In all cases, transcript accumulation broadly followed the patterns previously observed in B73 with respect to inoculation status and P addition (Fig. S7; Supporting Information). To synthesize growth, physiological and molecular data (Table 2), pairwise trait correlations were investigated across all treatments (Fig. 5a). Significant

298 positive correlations ($p < 0.05$) were observed between the abundance of fungal structures
299 and the accumulation of transcripts encoded by *RiEF, AM3* (respectively, fungal and plant
300 marker transcripts) and *Pt11*, *Pt13a*, supporting the primary role of these two transporters in
301 AM symbiosis. Accumulation of *Pt6* and *Pt13b* transcripts was significantly negatively
302 correlated ($p < 0.05$) with P accumulation and dry weight in both roots and shoots, indicating
303 induction under low-P stress. To investigate further patterns associated with AM outcome, the
304 analysis was repeated restricting the dataset to observations of M plants under low-P (Fig.
305 5b). A significant positive correlation ($p < 0.05$) was observed between the extent of root
306 external hyphae and P uptake from the hyphal compartment (PHC), which in turn was
307 positively correlated with shoot P and plant growth. Interestingly, the abundance of intra-
308 radical fungal structures was strongly, if not significantly, negatively correlated with both the
309 extent of root-external hyphae and PHC. At the molecular level, accumulation of *Pt6* and
310 *Pt8b* transcripts was significantly positively correlated ($p < 0.05$) with root dry weight, and,
311 at lesser significance, with SDW, root and shoot P content and PHC. Accumulation of *Pt13a*
312 transcripts showed also a weakly significant ($p < 0.1$) positive correlation with shoot dry
313 weight. Trait correlation in M plants at low-P was investigated further using principal
314 component (PC) analysis. Collectively, the first two PCs captured 76% of the trait variation
315 and well separated the six lines (Fig. 6). The abundance of root-external hyphae, PHC and
316 shoot P were observed to increase together with root and shoot dry weight and accumulation
317 of *Pt6* and *Pt8b.* Accumulation of the *Pt6* and *Pt8b* transcripts was more tightly associated
318 with increasing root and shoot dry weight than with root-external hyphae or PHC. Oh43 was
319 characterized by high levels of root-external hyphae, PHC, shoot P and MR. The line Pa36
320 was associated more with higher dry weight than higher PHC. Total AM colonization and

arbuscule abundance was antagonistic to the abundance of root-external hyphae, Hp301 and Mo18W exhibiting high levels of intra-radical fungal structures, but low levels of root-external hyphae, P accumulation, and root and shoot dry weight. Mo17 was distinct in expressing high levels of accumulation of AM associated transcripts, although with no associated increase in colonization, development of root-external hyphae or MR.

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) distinguished those that were highly responsive on the basis of poor performance in the absence of symbiosis (*e.g.* HP301) from those that benefited more from the symbiosis *per se* (*e.g.* Oh43). Support for this initial interpretation was obtained by detailed physiological characterization that provided a mechanistic explanation, and linked superior responsiveness to enhanced PAE on the basis of greater abundance of root-external hyphae.

P limitation of plant growth results primarily from the low mobility of P in the soil, especially at non-neutral pH, with AM fungi acting primarily to increase PAE through enhanced soil foraging (Smith & Read, 2008). Indeed, in this study, it was observed that increased plant growth in AM colonized plants was accompanied by greater P uptake. On reaching the root surface, or being delivered to the peri-arbuscular space, P will be rapidly taken up through the action of high-affinity PHT1 phosphate transporters, not limiting PAE. In contrast, movement of P into the vicinity of the root will be slow, resulting in the formation of a zone of P depletion surrounding the root (Bucher, 2007). As is consistent, it was not the extent of intra-radical colonization nor the accumulation of *Pt11* transcripts that showed the

344 greatest correlation with P uptake, but the abundance of root-external hyphae. If anything,
345 greater arbuscule abundance was negatively correlated with MR, HP301 and Mo18W
346 exhibiting high levels of colonization but low levels of AM P uptake. This is in general
347 support of previous studies (*e.g.* Schweiger & Jakobsen 1999, Jakobsen *et al.*, 2001, Yao *et*
348 *al.*, 2001, Schnepf *et al.*, 2008), including an evaluation of diverse fungal isolates with a
349 common plant host, which again found a similar correlation between fungal P uptake and
350 hyphal length (Munkvold *et al.*, 2004). Although a further report characterizing AM response
351 variation in four Chinese maize varieties (Chu *et al.*, 2013) did not reveal a clear relationship
352 between P uptake and the length of root-external hyphae, this may be a function of the
353 specific genotypes evaluated, of the fact that the contribution of mycorrhizal P uptake was not
354 directly quantified. Regarding the apparent antagonism between the abundance of intra-
355 radical and root-external fungal structures, the data are consistent with a trade-off in fungal
356 growth, the balance of which is influenced by plant genetic factors. Given the importance of
357 hyphal abundance to PUE, the data suggests that quantification of intra-radical structures
358 alone is not predictive of MR, nor an indication of the strength of mutualism.
359 Prior physiological characterization has demonstrated that AM P uptake is not a simple
360 addition to the plant's direct uptake pathway, but may represent a functional alternative: in the
361 extreme case, a colonized plant may obtain nearly all of its P requirement via the AM
362 pathway, whether as a result of down regulation of the direct pathway or owing to a greater
363 efficiency of fungal P foraging compared with that of plant roots (Smith *et al.*, 2003; Schnepf
364 *et al.*, 2008). Furthermore, the AM pathway may remain important at higher P availability,
365 even when MR itself is small, or even negative. In the evaluation of six selected lines,
366 increasing the availability of P resulted in reduced abundance of both root-external and intra-

radical fungal structures, along with declining MR. Nonetheless, the absolute quantity of P obtained via the AM pathway was greater, presumably as the result of increased Pi in solution as the capacity of the soil to adsorb P was saturated. At highest availability, in the lines with the highest P uptake, the growth response to shoot P accumulation was apparently saturated. B73 and Pa36 attained maximum growth irrespective of AM inoculation, although in both M plants accumulated less shoot P in the shoots than NC plants, *i.e.* they exhibited greater PUE. Interestingly, although at high P B73 and Pa36 showed equivalent levels of intra-radical fungal structures, the abundance of the root-external hyphae was greater in B73. Indeed, B73 supported a 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 illustrate the complexity of determining symbiotic outcome, and the range of plasticity among even six lines, with respect to just a single environmental variable.

Previous transcriptome profiling has identified rice marker genes whose mRNA accumulation correlates well with the establishment and development of AM symbiosis, well differentiating colonized from non-colonized plants (*e.g.* Guimil *et al*., 2005; Gutjahr *et al*., 2015). In this study, transcripts encoding PHT1 phosphate transporters were quantified not only to generalize among NC and M plants, but also to investigate differences in AM outcome among lines. Overall, AM colonization was positively correlated with the accumulation of transcripts encoded by *Pt11*, and to a lesser extent those encoded by *Pt13a, Pt13b* and *Pt14*, consistent with previous reports (Nagy *et al.*, 2006; Willmann *et al.*, 2013; Liu *et al*., 2016). Arbuscule abundance was found to be a poor predictor of MR among the six lines evaluated in this study. It is therefore consistent that variation in *ZmPt11* transcript accumulation was also largely independent of differences MR. These observations are

consistent with the physiological data suggesting P transfer at the peri-arbuscular interface to be non-limiting, either with respect to arbuscule abundance or to the concentration of PT11 proteins in the peri-arbuscular membrane. PT11 protein, however, has been reported also to regulate developmental responses to P limitation (Volpe *et al*., 2016), and high levels of *Pt11* accumulation may have additional significance beyond P transfer. A number of additional maize *Pt* transcripts responded to AM inoculation, although they were less abundant than those encoded by *Pt11.* Significantly, at low P, a mild positive correlation was observed between accumulation of *Pt13* transcripts and shoot biomass among colonized plants, indicating a role in the regulation of the symbiosis. Accumulation of *Pt6* and *Pt8b* transcripts, although generally lower in M relative to NC plants, was positively correlated with root and shoot dry weight among M lines. Accumulation of *Pt8b* was positively correlated also with P uptake from the hyphal compartment. Interestingly, the correlation was stronger with dry weight than P content, indicating this to be more than a secondary effect of differences in P accumulation. These observations, along with previous characterization of *pt11* and *pt13* mutants in rice (Yang *et al*., 2012), suggest a role for PHT1 proteins not only in P uptake but in the fine tuning of cost-benefit in AM symbioses.

Previous characterization of variation in MR has placed an emphasis on the development of intra-radical fungal structures, and marker transcripts have been identified allowing molecular-based quantification of intra-radical colonization. In this study, it was observed that variation in the abundance of root-external hyphae was more significant than levels of intra-radical colonization in determining AM outcome. Although accumulation of the well characterized *Pt11* transcript was not predictive of the abundance of extra-radical hyphae, correlations were observed between transcripts encoded by other *Pt* genes,

413 abundance of extra-radical hyphae and AM outcome. The identification of such variation,
414 coupled with the availability of NAM populations for quantitative trait loci mapping
415 (McMullen *et al.*, 2009), opens up the possibility to characterize the genetic basis of host
416 effects on the development of extra-radical hyphae, and potentially to develop molecular
417 breeding strategies to target this important, but hard to evaluate, component of MR.

418

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426

427 **AUTHOR CONTRIBUTION**

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

Table 1. Mycorrhiza response in diverse maize lines

	Dry mass (g)			Leaf P (ppm +/- SE)	
Line	NC	M	MR (95% CI)	NC	M
Oh43	0.96	2.81	1.85 (1.31 ,2.38)	292 +/- 42	625 +/- 72
CML322	1.13	2.59	1.47 (0.8 ,2.14)	375 +/- 47	489 +/- 3
HP301	0.44	1.82	1.37 (0.91 ,1.84)	475 +/- 50	383 +/- 106
A188	0.98	2.3	1.32 (1.04 ,1.61)	598 +/- 102	537 +/- 46
W64	1.02	2.34	1.32 (0.91 ,1.73)	341	427 +/- 107
B97	0.74	2.04	1.31 (0.89 ,1.73)	455 +/- 64	546 +/- 12
NC350	0.86	2.17	1.31 (0.8 ,1.81)	374 +/- 43	553 +/- 61
M162W	1	2.23	1.23 (0.86 ,1.61)	321	322 +/- 32
P39GB	0.87	2.11	1.23 (0.79 ,1.67)	289 +/- 38	325 +/- 54
Pa36	1.67	2.91	1.23 (0.71 ,1.76)	469 +/- 13	572 +/- 62
Ms71	1.4	2.62	1.22 (0.71 ,1.73)	376 +/- 60	571 +/- 41
CML333	1.19	2.4	1.2 (0.8 ,1.61)	327 +/- 25	546 +/- 77
Mo17	1.53	2.7	1.17 (0.7 ,1.64)	490 +/- 34	548 +/- 46
CML103	1.37	2.52	1.15 (0.87 ,1.43)	294 +/- 69	493 +/- 48
CML52	0.81	1.9	1.1 (0.7 ,1.49)	285 +/- 68	429 +/- 31
Ki11	1.02	2.1	1.08 (0.75 ,1.42)	283 +/- 35	396 +/- 66
IL14H	0.96	2.03	1.07 (0.8 ,1.34)	375 +/- 47	353 +/- 108
B73	0.78	1.82	1.05 (0.78 ,1.31)	498 +/- 113	364 +/- 80
CML277	0.93	1.96	1.03 (0.6 ,1.45)	416 +/- 90	468 +/- 43
CML228	1.38	2.35	0.97 (0.66 ,1.27)	396 +/- 43	403 +/- 100
M37W	0.94	1.91	0.97 (0.38 ,1.57)	390 +/- 37	535 +/- 117
CML247	1.08	1.98	0.9 (0.62 ,1.18)	334 +/- 71	398 +/- 9
Tx303	0.7	1.61	0.9 (0.46 ,1.34)	423 +/- 32	443 +/- 18
Ki3	0.94	1.79	0.85 (0.53 ,1.16)	496 +/- 25	401 +/- 50
Ky21	1.23	2.08	0.84 (0.49 ,1.19)	350 +/- 74	363 +/- 28
NC358	1.22	2.03	0.8 (0.42 ,1.19)	414 +/- 39	393 +/- 62
W22	1.15	1.93	0.78 (0.43 ,1.13)	480 +/- 100	465 +/- 101
Oh7b	1.05	1.81	0.76 (0.39 ,1.13)	236 +/- 26	550 +/- 78
Tzi8	1.48	2.24	0.76 (0.38 ,1.15)	340 +/- 24	340 +/- 34
Mo18W	0.94	1.66	0.72 (0.44 ,1)	393 +/- 39	422 +/- 31

Dry mass (g) and phosphorus (P) accumulation (ppm) in non-colonized (NC) and mycorrhizal (M) plants from the evaluation of 30 lines. Mycorrhiza response (MR) was calculated as M-NC, numbers in parentheses report a 95% confidence interval (CI). P accumulation reported +/- one standard error (SE).

Table 2. Summary of growth, physiological and molecular data

P	AMF	Line	SDW (g)	Shoot P (mg)	RDW (g)	Root P (mg)	Col Tot (%)	Col Arb (%)	Hyp (m/g)	PHC (mg)	*Pt6*	*Pt8b*	*Pt11*	*Pt13a*	*Pt13b*	*Am3*	*RiEF*
High	NC	B73	30	76	12	17	na	na	na	na	0.5	0.7	0.1	0	0	0	0
		Mo17	25	63	7	11	na	na	na	na	0.6	0.8	0	0	0	0	0
		HP301	28	67	9	14	na	na	na	na	0.4	0.4	0.3	0.1	0	0	0
		Pa36	30	93	7	9	na	na	na	na	0.5	0.7	0.3	0	0	0	0
		Mo18W	25	63	10	14	na	na	na	na	0.6	0.7	0.2	0	0	0	0
		Oh43	31	74	12	13	na	na	na	na	0.5	0.7	0.3	0	0	0	0
	M	B73	31	67	11	18	87	43	14.3	1.4	0.5	0.8	0.9	0.2	0.1	1	0.6
		Mo17	26	66	7	12	87	54	10.6	0.7	0.5	0.7	0.8	0.2	0.2	0.9	0.7
		HP301	21	50	6	11	69	43	7.8	0.6	0.5	0.4	0.8	0.3	0.2	1	0.5
		Pa36	31	69	10	12	88	54	9.3	1	0.5	0.6	0.9	0.3	0.3	1	0.5
		Mo18W	22	59	7	12	84	56	10.2	0.8	0.5	0.7	0.8	0.3	0.2	0.9	0.2
		Oh43	27	69	9	12	75	45	10.3	0.8	0.5	0.7	0.8	0.2	0.1	1	0.4
Low	NC	B73	8	10	3	4	na	na	na	na	0.8	0.8	0.6	0.2	0.3	0	0
		Mo17	12	17	4	3	na	na	na	na	1.1	0.9	0.7	0.5	0.6	0	0
		HP301	4	6	1	1	na	na	na	na	0.9	0.5	0.6	0.3	0.4	0	0
		Pa36	14	17	6	3	na	na	na	na	0.9	0.8	0.6	0.4	0.4	0	0
		Mo18W	4	6	2	2	na	na	na	na	0.9	0.8	0.7	0.2	0.4	0	0
		Oh43	6	9	3	2	na	na	na	na	0.9	0.8	0.6	0.2	0.4	0	0
	M	B73	17	34	5	6	93	85	16.9	0.4	0.7	0.7	1	0.4	0.4	1.1	0.5
		Mo17	19	34	5	7	88	79	13	0.4	0.8	0.9	1.3	0.6	0.6	1.4	0.7
		HP301	14	28	3	5	93	78	10.5	0.2	0.6	0.4	0.9	0.4	0.4	1	0.5
		Pa36	22	40	6	6	94	83	14.2	0.4	0.7	0.7	1	0.5	0.5	1.1	0.6
		Mo18W	15	32	5	7	95	79	11.3	0.3	0.7	0.7	0.9	0.4	0.4	1	0.5
		Oh43	19	39	5	6	88	70	21.4	0.5	0.7	0.8	1	0.4	0.4	1.1	0.5

Characterization of colonized (M) and non-colonized (NC) plants of sixe selected lines grown under high (53.2 mgP/Kg) or low (7.9 mgP/Kg) phosphorus (P) availability. SDW, shoot dry weight; shoot P, P accumulation in the shoot; RDW, root dry weight, root P, P accumulation in the root; Col Tot, abundance of intra-radical fungal structures; Col Arb, arbuscule abundance; HYP, length density of root-external hyphae, PHC, P uptake from the hyphal compartment; *Pt6*, *Pt8b*, *Pt11*, *Pt13a*, *Pt13b*, *Am3* and *RiEF,* transcript accumulation normalized to

FIGURE LEGENDS

Fig. 1. Mycorrhiza response is correlated with enhanced P uptake. (a) Shoot dry weight (SDW, g; normalized with respect to differences among replicates) of 30 diverse maize lines grown for 8 weeks, under greenhouse conditions, with (M; right box) or without (NC left box) inoculation with the fungus *Funneliformis 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 mean values of NC (1.05g, n=540) and M (2.16g, n=552) groups are shown by vertical lines. Lines ordered by increasing mycorrhizal response from top to bottom. Box shading indicates mean accumulation of P^{31} (ppm) in the shoot as determined by ionomic analysis, colour-key shown at top of panel. (b) Reaction norms for 30 diverse maize lines contrasting shoot dry-weight (R SDW, g; residual SDW with respect to group mean) of non-inoculated plants (NC) and plants inoculated with the fungus *Funneliformis mosseae* (M). Segments corresponding to six lines selected for further study are labeled and shown in bold. Point shading indicates accumulation of P31 (ppm) in the shoot as (a). (c) Accumulation of phosphate in leaves (P^{31}, ppm) and shoot dry weight (SDW, g) in the 30 maize lines (points correspond to mean values). Linear fit (yellow line) and associated 95% confidence interval (shaded area) shown.

Fig. 2. Shoot dry weight (Shoot DW; g) is correlated with total phosphorus (P) accumulation (Shoot P; mg) in six maize genotypes grown with (yellow circles) or without (open circles) inoculation with *Rhizophagus irregularis*, under three P regimes (7.9 mgP/kg, 15.5 mgP/kg, 53.2 mgP/kg). Points indicate the mean of n observations; whiskers extend +/- 1 standard error; trend lines based on a linear fit to individual observations for each treatment.

Fig. 3. Phosphorus (P) accumulation is correlated with the abundance of extra-radical hyphae. (a) percentage of total root length containing mycorrhizal structures (brown) and arbuscules (yellow) observed in inoculated seedlings of six maize inbred lines, grown under three different levels of phosphorus abundance (7.9, 15.5 and 53.2 mgP Kg^{-1}). Boxes show 1st quartile, median and 3rd quartile. Whiskers extend to the most extreme points within 1.5x box length. (b) Shoot phosphate acquired from ^{33}P soil (mg) as a function of the length of external hyphal (m g^{-1} soil).

Fig. 4. Accumulation of transcripts encoding PHT1 proteins responds to phosphorus availability and AM colonization. Heatmap representation of accumulation of selected *ZmPt* (*Pt*) transcripts quantified by real-time PCR relative to *beta-actin.* Accumulation of selected *Pt* transcripts was quantified in B73 seedling shoot or root tissue. Plants were grown across a range of increasing P availability at 10μM, 100μM, 1000μM P without inoculation with AMF (NC) and also at 100μM with inoculation with *R. irregularis* at 100μM (M). Mean accumulation was determined from three biological replicates, standardized (Z-score) within transcripts across experimental treatments and represented on a scale from white (below average accumulation) to brown (above average accumulation). Accumulation of the maize mycorrhizal marker transcript *Am3* and the *R. irregularis* elongation factor *RiEF* is also shown. Transcripts responsive to inoculation with AMF marked with an asterisk.

Fig. 5. Accumulation of transcripts encoding PHT1 protiens is correlated with phosphorus accumulation and AMF inoculation across diverse maize lines. Selected *ZmPt* (*Pt*) transcripts, the maize mycorrhizal marker transcript *Am3* and the *R. irregularis* elongation factor *RiEF* were quantified by real-time PCR in samples taken from the roots of plants described in Fig. 2. (a) Correlation matrix (Pearson) of transcript accumulation, shoot and root dry weight (DW, g), shoot and root phosphorus accumulation (P, mg), proportion of root length colonized (% Col, all structures) and proportion of root

length containing arbuscules (% Arb). Red circles indicate positive correlation; blue circles indicate negative correlation; the size and intensity of shading indicate magnitude. Correlations significant at $p < 0.05$ marked with an asterisk. Correlations significant at $0.05 < p < 0.1$ marked with a point. (b) as (a) with data restricted to observations of AM colonized plants grown under low P.

Fig. 6. AM P uptake is correlated with the extent of the root-external mycelium under low P. Principle component analysis (PCA) of plant-growth, physiological and molecular observations of inoculated plants grown under low P. Biplot showing scores in the first two principal components (PC1, PC2) 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), transcript accumulation of *Pt6*, *Pt8b*, *Pt11*, *Pt13a*, *Pt13b*, *Am3* and *RiEF*) and the individual genotypes (B73, Mo17, HP301, Pa36, Mo18W, Oh43). Points indicating the different genotypes are colour-coded by mycorrhizal response (MR, g) calculated as the difference in shoot dry weight in colonized and non-colonized plants.

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SUPPORTING INFORMATION

The following Supporting Information is available for this article:

Fig. S1 Association with mycorrhizal fungi promotes vegetative growth in diverse maize lines

Fig. S2 Inoculation with AMF is associated with changes in the ionome

Fig. S3 Oh43 and Mo18W differ in elemental profile

Fig. S4 Shoot dry weight is correlated with seed size

Fig. S5 Phylogeny of maize PHT1 proteins

Fig. S6 Maize *PHT1* transcripts accumulate throughout the plant life-cycle

Fig. S7 Accumulation of six *ZmPt* (*Pt*) transcripts in root-samples

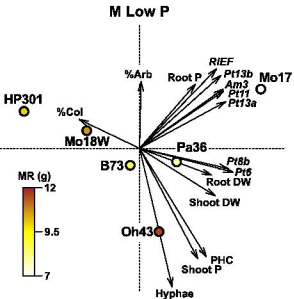
Table S1 Element accumulation in roots and leaves of non-inoculated and inoculated plants

Table S2 Phosphorus transfer data at low P

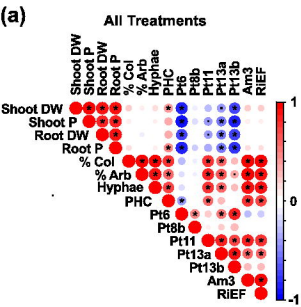
Table S3 *Pht1* genes of maize (var. B73)

Table S4 Primers used in this study

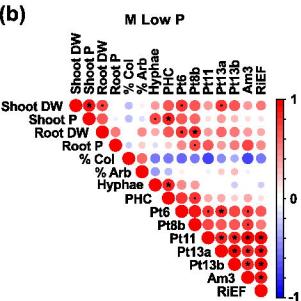
Supplementary Analysis

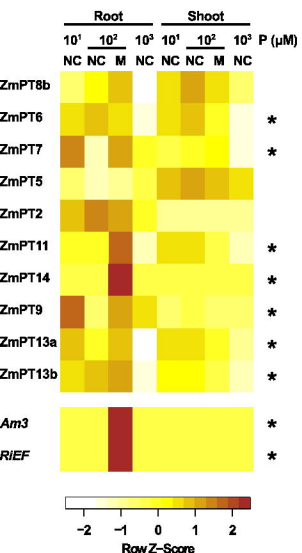

M Low P
%Arb
Root P
RIEF
Pt13b
Am3
Pt11
Pt13a
Mo17
HP301
%Col
Mo18W
Pa36
B73
Pt8b
Pt6
Root DW
Shoot DW
MR (g)
12
9.5
7
Oh43
PHC
Shoot P
Hyphae

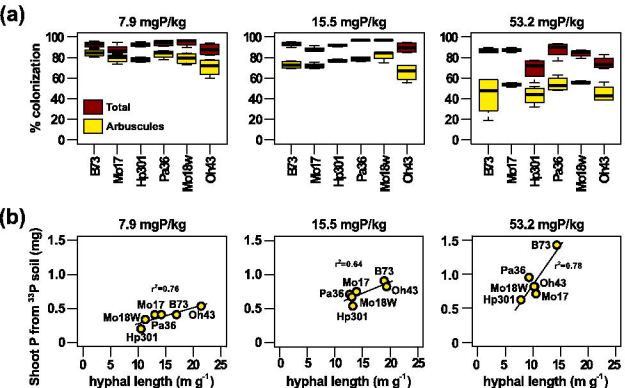
(a)

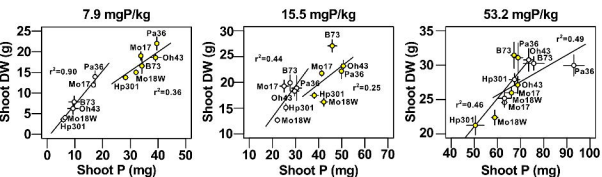

All Treatments
Shoot DW
Shoot P
Root DW
Root P
% Col
% Arb
Hyphae
PHC
Pt6
Pt8b
Pt11
Pt13a
Pt13b
Am3
RIEF
1
0
-1

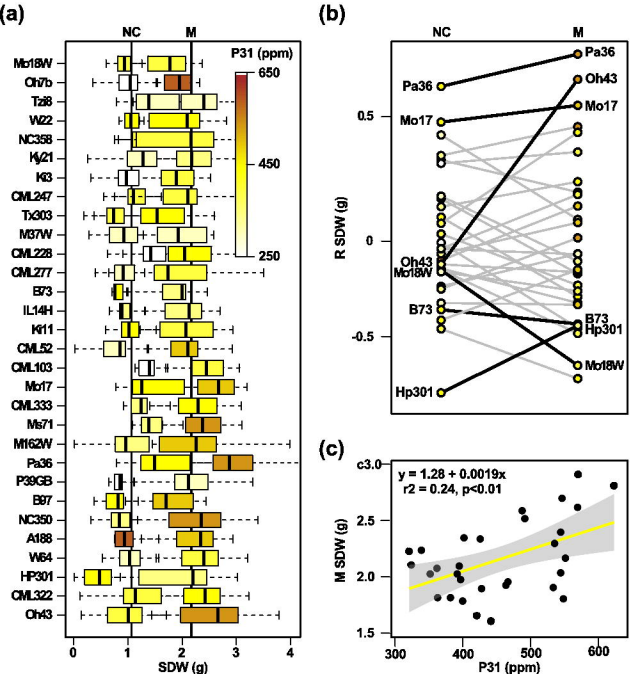
(b)


M Low P
Shoot DW
Shoot P
Root DW
Root P
% Col
% Arb
Hyphae
PHC
Pt6
Pt8b
Pt11
Pt13a
Pt13b
Am3
RIEF
1
0
-1


Root
Shoot
10¹ 10² 10³ 10¹ 10² 10³ P (μM)
NC NC M NC NC NC M NC
ZmPT8b
ZmPT6
ZmPT7
ZmPT5
ZmPT2
ZmPT11
ZmPT14
ZmPT9
ZmPT13a
ZmPT13b
Am3
RIEF
*
−2 −1 0 1 2
Row Z−Score


(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


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)
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
Mo17
Oh43
Mo18W
B73
Hp301
Pa36
Oh43
Mo17
B73
Hp301
Mo18W
0.5
0
-0.5
R SDW (g)
(c)
y = 1.28 + 0.0019x
r2 = 0.24, p<0.01
c3.0
2.5
2.0
1.5
M SDW (g)
300
400
500
600
P31 (ppm)