Short title: Outcome variation in maize mycorrhizal symbiosis

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: rsawers@langebio.cinvestav.mx

Full title: Outcome variation in maize interaction with arbuscular mycorrhizal fungi is correlated with the extent of extra-radical mycelium

Ruairidh J. H. Sawers[1,2*], Simon F. Svane[3,4], Clement Quan[5], Mette Grønlund[3,4], Barbara Wozniak[2], Mesfin Nigussie Gebreselassie[2], Matthias Mueller[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, México.
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

Summary: Corn varieties that promote greater extra-radical growth of symbiotic arbuscular mycorrhizal derive greater benefit from the interaction

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Corresponding author e-mail: rsawers@langebio.cinvestav.mx

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## ABSTRACT

In light of the rising cost and often limited access to agricultural fertilizers, arbuscular mycorrhizas are attracting ever greater interest for their potential to promote more efficient use of the world's mineral resources. This potential remains largely unrealized, in part because of a lack of understanding of the factors determining the outcome of the symbiosis in any given context, and it remains to be demonstrated to what extent host genetic variation can drive the symbiosis in the direction of greater plant benefit under agricultural conditions. In this work, a panel of maize inbred lines, selected to maximize genetic diversity, was evaluated with and without inoculation with mycorrhizal fungi. In addition to measuring plant growth, fungal morphology, transfer of phosphorus to the plant, plant element profiles and accumulation of transcripts encoding the PHT1 family of plant phosphate transporters were also characterized. The relative performance of lines changed between non-inoculated and inoculated plants and it is proposed that such genotype x inoculation interaction is indicative of variation in host capacity to profit from symbiosis *per se,* and not only the result of differences in tolerance of low phosphate availability. The greatest growth response, observed in the line Oh43, was correlated with low arbuscule abundance but extensive development of extra-radical mycelium and fungal P transfer. The data suggest that host genetic factors influence fungal growth strategy with subsequent impact on the outcome of the symbiosis.

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## INTRODUCTION

The rising cost of agricultural inputs and an increasing awareness of the negative environmental consequences of their intensive use fuel an ever greater interest in beneficial crop-microbe interactions and their potential application (Perez-Montano et al., 2014; Vance, 2014). The most prevalent nutrient-delivering plant-microbe interaction is the association with fungi of the phylum *Glomeromycota*, resulting in the formation of arbuscular mycorrhizal symbioses (Parniske, 2008). More than 80% of extant terrestrial plants establish arbuscular mycorrhizal (AM) associations, and this fundamental capacity has been retained in the 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 establishment of AM symbioses and nutrient exchange (Paszkowski et al., 2002; Gutjahr et al., 2008).

AM fungi provide the plant host with greater access to soil nutrients and water through connection to a network of fungal hyphae that extends beyond the root itself (Bucher, 2007). In addition, AM symbioses have been implicated in enhanced tolerance to a range of abiotic and biotic stresses (Smith and 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 mycorrhizal 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, although such improvement need not indicate a superior capacity to benefit from colonization *per se* (Sawers et al., 2010) - and the question remains as to whether 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 and 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

117 greater soil volume. Physiological studies have demonstrated that symbiotic phosphate
118 uptake is a distinct functional alternative to direct uptake by the plant (Smith et al., 2003;
119 Bucher, 2007)[ ]. Significantly, it has been suggested that in a field setting the majority of
120 the phosphate taken up by a plant may be acquired via the symbiotic route (Smith et al.,
121 2003; Yang et al., 2012)[ ].

122 Molecular analyses have further defined the distinction between symbiotic and direct
123 phosphate uptake through characterization of the plant PHT1 proton:phosphate symporters
124 (Bucher, 2007). In all plants characterized to date, the PHT1 family consists of multiple
125 proteins playing diverse roles in P uptake and internal transport, and, where plants are
126 competent to host AMF, there is at least one family member that can be postulated to act
127 predominantly, or exclusively, under symbiosis (Rausch et al., 2001; Harrison et al., 2002;
128 Paszkowski et al., 2002; Nagy et al., 2005[ ]; Glassop et al., 2005; Maeda et al., 2006; Caesar
129 et al., 2014; Walder et al., 2015). The best characterized mycorrhiza-associated PHT1
130 proteins are associated with the peri-arbuscular membrane and, interestingly, have been
131 shown by mutant analysis to be indispensable for arbuscule development under standard
132 conditions in pot experiments (Maeda et al., 2006; Javot et al., 2007, Yang et al., 2012,
133 Willmann et al., 2013).

134 In this study, differences in mycorrhiza response among a panel of diverse maize lines
135 are dissected to identify variation linked to a greater ability of the host to profit from the
136 symbiosis. In a first screen, a panel of 30 diverse maize lines, consisting of the 26 parents of
137 the maize Nested Association Mapping (NAM) population (McMullen et al., 2009), a
138 collection selected to maximize genetic diversity from global breeding germplasm, and a
139 small number of additional genotypes, were evaluated in a greenhouse small-pot experiment.

On the basis of the first screen, a pair of lines were identified that while similar in performance under non-inoculated conditions differed greatly when colonized. This selected pair of lines and four further lines of interest were further analysed by measurement of P uptake, quantitative characterization of fungal colonization and quantification of transcripts encoding PHT1 family P transporters. Data suggest that plant host factors can manipulate the pattern of fungal growth with a significant impact on symbiotic outcome.

## RESULTS

### Diverse maize lines show a range of outcomes under AM symbiosis

To define physiological and molecular patterns correlated with variation in the outcome of AM symbioses, a panel of diverse maize lines, consisting of the 26 diverse inbred founders of the maize NAM population (McMullen et al., 2009) , Pa36 (a P efficient line; Kaeppler et al., 2000), B73 (the genome sequence reference line), W22 (a line widely used in reverse-genetics) and W64A (a line used previously for study of AM symbiosis; Paszkowski et al., 2006), was evaluated under greenhouse conditions in a sand/loam substrate, under P limiting conditions, with (M) or without (NC) inoculation with the fungus *Funneliformis mosseae*. Eight weeks after emergence, plants were harvested and shoot dry-weight (SDW; g) determined (Table 1). Over the period 2007 – 2010, six experiments (Exp A-F) were grown in randomized block designs, each consisting of 3 replicates, with the exception of Exp D that consisted of 5 replicates. Data were combined across plantings and normalized with respect to differences among experiments (see Materials and Methods and Supplemental Material).

Collectively, the evaluated lines showed a positive outcome when inoculated with AMF (Fig. 1), with a significant ($p<0.001$, *t*-test) increase in mean SDW from 1.05g in NC

plants to 2.16g in M plants, equating to a panel-wide mycorrhiza response (MR = M - NC; Sawers et al., 2010) of 1.1g ±0.08g (95% interval for difference in means). For experiments A and C, roots were harvested and the abundance of fungal structures quantified by microscopic inspection. NC plants were confirmed to be free of fungal structures. M plants were well colonized, 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%). At the level of individual lines, all showed increased SDW following inoculation with AMF (Fig. 2, Fig. 3; Table 1). Estimates for mean SDW by line of NC plants ranged from 0.44g (Hp301) to 1.67g (Pa36), and for M plants from 1.61g (Tx303) to 2.91g (Pa36). Estimates of MR ranged from 0.72g (Mo18W) to 1.85g (Oh43). In no instance did the 95% confidence interval for MR include 0 (Fig. 2, upper panel), indicating a significant positive outcome of symbiosis in all lines. In the panel evaluation, the line Oh43 was the most highly responsive (highest point estimate of MR; 95% confidence interval for MR did not include the panel-wide MR mean), and the line Mo18W the least responsive (lowest point estimate of MR; 95% confidence interval for MR did not include the panel-wide MR mean). On the basis of non-overlapping MR intervals, the line Oh43 was significantly more responsive than the lines Mo18W, Oh7b, Tzi8, W22, NC358, Ky21, Ki3 and CML247. As a first approximation to account for dependence differences (Janos 2007; Sawers et al., 2010), the reaction norm of M relative to NC plants was examined (Fig. 3). The contrast in outcome in Oh43 and Mo18W was clearly reflected by shifting performance relative to other lines in the panel: the two lines were very similar and typical of the panel as a whole when non-inoculated, but outlying following inoculation

(Fig. 3); *i.e.* Oh43 and Mo18W illustrate a pattern of SDW consistent with similar dependence but divergent capacity to profit from AM symbiosis.

**Leaf and root elemental profiles change in response to colonization by AMF**

Given that plants were evaluated under P limiting conditions with all other nutrients provided in excess, it was anticipated that variation in performance would be driven largely by differences in P accumulation. To test this hypothesis and more broadly assess plant nutrition, root and leaf samples were analyzed further using inductively coupled plasma-mass spectroscopy (ICP-MS; Baxter et al., 2008) to quantify the accumulation of P along with nineteen other elements. Pooling data across lines, ten quantified elements (Na, Al, P, S, Mn, Fe, Co, Ni, Zn, Cd) were found to accumulate to different levels in M compared with NC plants, in either roots and/or leaves ($p<0.05$, adjusted for multiple tests; Fig. 4; Table 2). Accumulation of these ten elements was analyzed by principal component (PC) analysis. The first three PCs explained 75%, 8% and 7% of the variation in the accumulation of the ten selected ions, respectively, PC1 being dominated by differences between roots and leaves. Inspection of PC loading with respect to the ten selected ions indicated the magnitude of the contribution to PC1 was approximately equal for all ions, although the sign of loading was different for P with respect to the others (Fig. 4B). Loading on PC2 and PC3 was largely related to P accumulation, with important additional contributions from Mn, S and Na (Fig. 4B; Fig. S1A;B). Plotting PC scores against ion accumulation revealed a mild tendency towards higher P accumulation at lower values of PC1, that was more marked with respect to PC2 and PC3 (Fig. 4C). A number of additional patterns were observed, including a tendency to higher accumulation of Mn and S at higher values of PC1, a trend opposite to that observed for P (Fig. 4C). To explore variation in elemental profiles further, PC scores were plotted by

sample (Fig. 5). PC1, again, strongly differentiated between roots and leaves, PC2 was largely related to variation in P accumulation, although not clearly distinguishing tissue type or inoculation status, while PC3 differentiated NC and M plants. PC scores corresponding to the lines Oh43 and Mo18W were examined in more detail (Fig. 5). In root samples, PC scores differed little between the two lines or between NC and M plants. In leaves, however, Mo18W samples showed similar PC scores in NC and M plants, while Oh43 samples exhibited a marked shift to lower scores in PCs 1, 2 and 3 when inoculated, concomitant with an increase in P accumulation that was not evident in Mo18W (P accumulation means groups, Tukey HSD, $\alpha$=0.1).

**Mycorrhizal response is correlated with the extent of the extra-radical mycelium**

Having established that the differential response of Oh43 and Mo18W to inoculation with AMF was correlated with a difference in P accumulation in the leaves, it was decided to directly characterize P uptake in these lines. Oh43 and Mo18W (along with the NC outlying lines Pa36 and HP301 and the reference lines B73 and Mo17 ) were grown in PVC-tubes (Fig. 6A) containing a fine mesh, allowing passage of hyphae but not plant roots (Smith et al., 2003), that separated the main soil volume (root-hyphae compartment; RHC) from a hyphae (HC) compartment containing soil mixed with $^{33}P$ at a specific activity (SA=$^{33}P/^{31}P$ ) of 144 kBq $mg^{-1}$ P. Plants were grown with or without inoculation with *Rhizophagus irregularis* and, at tassel emergence, harvested and P uptake determined (Table 3). In this experiment, all six genotypes exhibited increased biomass production upon inoculation, with Oh43 again showing the greatest SDW response (Fig. 6A, 6B). Here, however, the line Mo18W showed a greater response than previously, although this was rather driven by lower NC than by

231 greater M SDW, relative to the other lines, compared with the panel evaluation. Total shoot P
232 was measured and found to correlate well with SDW, both among lines and between NC and
233 M plants (Fig. 6C, 6D). Oh43 showed the greatest response in P accumulation to inoculation
234 with AMF, accumulating P to typical levels for non-inoculated plants, but to high levels when
235 inoculated, relative to the other lines in the experiment. Although more responsive to AMF,
236 when fungal structures were quantified, there was no evidence that Oh43 was colonized to a
237 greater extent than other lines; indeed, the frequency of arbuscules was marginally lower
238 (Fig. 7A). In contrast, Oh43 supported a significantly greater development of extra-radical
239 hyphae (Fig. 7B). Across all lines, the density of extra-radical mycelium correlated positively
240 with AM P uptake (Fig. 7B). Expressing the ratio of of $^{33}$P uptake from HC soil to the length
241 of roots containing arbuscules, Oh43 showed a significantly higher value than the other lines
242 evaluated (Fig. 7C).
243
244 **The B73 maize genome encodes 13 PHT1 phosphate transporters**
245 On the basis that performance variation was correlated with differences in P uptake and
246 accumulation, a molecular characterization of the maize *Pht1* phosphate transporter family
247 was performed. Thirteen maize *Pht1* genes were identified in a search of the B73 maize
248 genome. Accession numbers of the 13 genes are given in Table 4 along with a conventional
249 numbered annotation of the form *ZEAma;Pht1* (abbreviated from here on as *ZmPt*). Gene
250 numbers were assigned on the basis of the similarity to rice PTs (Paszkowski et al., 2002) .
251 Previous reports of maize genes are indicated in Table 3, but the previous annotation has not
252 necessarily been retained. In common with previously characterized *PT* genes (Karandashov
253 & Bucher, 2005) , the maize *Pt* genes are predicted to encode proteins containing 12

membrane-spanning helices, divided into two blocks by a long inter-helical loop between helices 6 and 7. Helix 4 contains a canonical motif GGDYPLSATIxSE (Karandashov and Bucher, 2005), in which for all of the maize proteins, the variable residue x is a methionine.

Maize and rice PT protein sequences were aligned , along with a number of previously characterized mycorrhiza-associated sequences from other species, and this alignment was used to construct a maximum likelihood tree, identifying a number of groups within the maize protein family (Fig. 8). The protein ZmPT11 (previously reported as ZmPHT1;6. Nagy et al., 2006; Willmann et al., 2013) was the unique maize member of a group including the well-characterized mycorrhiza-associated proteins medic MtPT4 (Harrison et al., 2002) , rice OsPT11 (Paszkowski et al., 2002), potato StPT4 (Nagy et al., 2005) and tomato LePT3 (Nagy et al., 2005). The maize protein ZmPT14 was the only maize member of a second mycorrhiza-associated group, grouping with the previously characterized proteins barley HvPT8 and wheat TaPT1:myc (Glassop et al., 2005) . The proteins ZmPT9 and ZmPT13a-d defined a further group along with the single mycorrhiza-associated rice protein OsPT13 (Yang et al., 2012) . The remaining ZmPT proteins belonged to a larger group that contained a number of rice proteins characterized previously to play roles in direct P uptake and translocation.

Four *ZmPt* genes are located on chromosome 1, four on chromosome 2, one on chromosome 5, two on chromosome 7, one on chromosome 8 and one on chromosome 10 (www.maizegdb.org; Sen et al., 2010). The genes *ZmPt5*, *ZmPt13a*, *ZmPt13c* and *ZmPt13d* are clustered within a ~500kb region of chromosome 2 adjacent to the centromere, the three *ZmPt13* sequences lying within a region of ~100kb. The gene *ZmPt13c* is identical to the gene *ZmPt13b* located on chromosome 7. The region of nucleotide identity extends up- and

277 downstream from *ZmPt13b/c* and it was not possible, therefore, to differentiate the two genes
278 in subsequent expression studies. Similarly, we cannot discount the possibility that the
279 sequences *ZmPt13b*/*c* represent a single gene, erroneously assigned to two locations in
280 genome assembly. In the case of a second pair of closely related sequences, *ZmPt8a* and
281 *ZmPt8b*, there was sufficient nucleotide variability to discriminate between them. The
282 position of *ZmPt8a* and *ZmPt8b* in the genome suggests that they are retained paralogs from
283 the tetraploid event that occurred in maize sometime after divergence from sorghum
284 (Schnable et al., 2011) .
285
286 ***ZmPt* transcripts show tissue and stage specific patterns of expression**
287 To gain a general panorama of PT function in maize, existing transcriptome datasets were
288 examined for evidence of the accumulation of *ZmPt* transcripts. Using data from two sources
289 profiling seedling tissues (Wang et al., 2009; Li et al., 2010) and one source profiling
290 reproductive tissues (Davidson et al., 2011) , transcripts encoded by representatives of the *Pt*
291 family were found in all tissues and developmental stages examined, indicating both the
292 importance on the *Pt* gene family throughout the plant life cycle and a specialization among
293 family members (Fig. S2). In vegetative tissues, the predominant *Pt* transcripts were encoded
294 by *ZmPt8a*, *ZmPt8b* and *ZmPt2*, all three transcripts accumulating to the greatest level in
295 seedling roots. The transcripts *ZmPt5* and *ZmPt13a* showed also significant accumulation in
296 vegetative tissues, although primarily in mature leaves. The transcripts *ZmPt6* and *ZmPt7*
297 exhibited specific accumulation in the tassel. Transcripts encoded by the other maize *Pt*
298 family members were not detected at significant levels in these data sets.
299

**Accumulation of *ZmPt* transcripts responds to inoculation with AMF**

Having used available transcriptomic data sets provided an indication of the function of maize *Pt* genes throughout the plant life-cycle, under nutrient replete conditions, and in the absence of microbial symbiosis, it was decided to investigate directly quantify transcript accumulation in AMF inoculated plants. Ten transcripts were selected and quantified 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. intraradices* inoculation . Seven of 10 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, $\alpha$=0.05; Fig. 9A). The transcripts *Pt7*, *Pt9*, *Pt11*, *Pt13a* and *Pt14* accumulated to significantly higher levels in the roots of M plants compared to NC plants under the same P availability. 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 under the same P availability. 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 not clearly correlated with symbiotic outcome**

With the maize *Pt* gene family defined and transcript accumulation characterized in B73, it was possible to investigate the potential relationship between AM outcome and PT transporter function. On the basis of the original characterization, four *ZmPt* genes (*Pt6*, *Pt8*, *Pt11* and *Pt13a*) were selected and accumulation of their transcripts quantified in root

samples collected from the phosphate-transfer experiments described above using the lines B73, Mo17, Hp301, Pa36, Mo18W and Oh43, grown under low- or high-P, with or without inoculation with *R. intraradices*. Transcripts encoded by *Pt6* accumulated to significantly lower levels in M plants in all lines (Tukey HSD, $\alpha$=0.05 ; Fig. 9B). In contrast, accumulation of *Pt11* and *Pt13a* was significantly increased in M plants (for a given P level and genotype, M and NC samples fell within different means groups with the exception of *Pt11* in Mo18W under low P, and *Pt13a* in Mo17 and Hp301 under low P; Tukey HSD, $\alpha$=0.05 ; Fig. 9B). With regard to variation among lines, the most striking differences were observed in Mo17, in which transcripts of all four *Pt* genes were observed to accumulate to higher levels than in the other lines under at least one experimental condition (*Pt11* and *P13a* in M plants under low P; *Pt6* in NC plants under low P; *Pt8b* in both NC and M plants under low P; Tukey HSD, $\alpha$=0.05 ; Fig. 9B). In addition, *Pt8b* transcripts accumulated to significantly lower levels in Hp301 than in all other lines, in all treatments (Tukey HSD, $\alpha$=0.05 ; Fig. 9B). With respect to the candidate lines Mo18W and Oh43, there was no indication of differential accumulation between the two genotypes for any of the *Pt* transcripts assayed (Tukey HSD, $\alpha$=0.05 ; Fig. 9B).

## DISCUSSION

Efforts to better employ mycorrhiza in agriculture demand a greater understanding of the molecular and physiological basis of variation in symbiotic outcome. In this study, AM outcome was evaluated in a panel of maize inbred lines selected to maximize genetic diversity, complemented by measurement of biomass production with characterization of

345 fungal structures, P uptake, elemental profiling, and quantification of transcripts encoding
346 PHT1 phosphate transporters. The study panel was composed primarily of the parents of the
347 maize Nested Association Mapping (NAM) population, a collection selected from global
348 breeding germplasm to maximize overall genetic diversity (McMullen et al., 2009). The
349 panel proved phenotypically diverse in greenhouse evaluation, and a range of plant
350 performance was observed in both NC and M plants. From the perspective of P efficiency, the
351 line Pa36 (not itself a NAM founder) was notable for presenting the highest biomass
352 production in both NC and M plants. With regard to variation in AM outcome, greatest
353 emphasis was placed on line x inoculation effects, and consequently the lines Mo18W and
354 Oh43 were selected for further study: these two lines showed similar performance in the
355 absence of inoculation while differing when inoculated. Concomitantly, their relative
356 performance with respect to the other lines changed between the non-inoculated and
357 inoculated trial. No clear correlation was evident between the abundance of intra-radical
358 fungal structures and biomass production. However, Oh43, the line showing the greatest
359 response to inoculation, supported the most extensive extra-radical mycelium, consistent with
360 the interpretation that the P contribution from the AM uptake pathway is determined largely
361 by hyphal abundance in the soil. This is in general support of several previous studies (see
362 e.g. Schweiger and Jakobsen 1999, Jakobsen et al., 2001, Yao et al., 2001, Schnepf et al.,
363 2008), including the correlation between fungal P uptake and hyphal abundance among 15
364 isolates of *Funneliformis mosseae* used to inoculate a common plant parent (Munkvold et al.,
365 2004). Although a report characterizing AM response variation in four Chinese maize
366 varieties (Chu et al., 2013) did not reveal a clear relationship between P uptake and the
367 length of root-external hyphae, this may be a result of the small number of genotypes

368 characterized. With regard to the specific case of Oh43 and Mo18W, it is concluded that
369 maize genotype contributes to variation in the extent of the hyphal network and as a
370 consequence to outcome variation in a given environmental setting.

371 A strong correlation was found between shoot P accumulation and plant biomass, both
372 among lines and between inoculated and non-inoculated plants. This observation suggests
373 that, under the P limiting conditions of the experiments, difference in P uptake was the major
374 driver of outcome variation. Physiological characterization has demonstrated that P uptake
375 via the AMF pathway is not a simple addition to the plant's own direct uptake pathway, but
376 may represent a functional alternative: in the extreme case, a colonized plant may obtain
377 nearly all of its P requirement via the AMF pathway, whether as a result of down regulation
378 of the direct pathway or owing to a greater efficiency of fungal P foraging compared with
379 plant roots (Smith et al., 2003; Schnepf et al., 2008). Here, the AMF pathway accounted
380 for 30% - 70% of the total P uptake in inoculated plants. Concomitantly, both up-regulated
381 *ZmPt* transcripts ( *Pt11* and *Pt13a* in roots the analysis of diverse lines; *Pt7*, *Pt9*, *Pt11*, *Pt13a*
382 and *Pt14* in roots in the analysis of B73) and down-regulated *ZmPt* transcripts (*Pt6* in roots in
383 our analysis of diverse lines; *Pt6*, *Pt9*, *Pt11*, *Pt13a* and *Pt13b* in leaves in our analysis of
384 B73) were identified following inoculation, consistent with the shift in the pathway of P
385 uptake. In medic, trefoil and rice, transcripts encoded by orthologs of *ZmPt11* (*MtPT4,*
386 *LjPT4* and *OsPT11*, respectively) accumulate uniquely in the roots of plants colonized by
387 AMF (Harrison et al., 2002; Paszkowski et al., 2002; Maeda et al., 2006). Furthermore,
388 mutant analysis has demonstrated the proteins encoded by these transcripts to be essential for
389 formation and maintenance of AM symbiosis under nutrient replete conditions in pot
390 experiments (Maeda et al., 2006; Javot et al., 2007; Yang et al., 2012). The protein MtPT4 has

391 been localized to the peri-arbuscular membrane (Harrison et al,. 2002; Kobae and Hata, 2010;
392 Pumplin et al., 2012), consistent with the hypothesis that this divergent group, encoded by
393 single copy genes in the characterized plant species, provide the principal route of P uptake
394 from the periarbuscular space to the plant. Here, it was observed that although *Pt11*
395 transcripts did, as expected, accumulate to high levels in colonized roots, there was
396 significant accumulation also in the roots and shoots of NC plants, consistent with previous
397 reports (Nagy et al., 2006; Willmann et al., 2013) and similar to reports of orthologous genes
398 in brachypodium, foxtail millet and sorghum (Hong et al., 2012; Ceasar et al., 2014; Walder
399 et al., 2015). Interestingly, although a maize *pt11* mutant has been reported to reduce
400 formation of mycorrhizas in greenhouse experiments, colonization was restored in the field or
401 by co-cultivation with mycorrhizal nurse plants, although plant performance was poor
402 (Willmann et al., 2013). The analysis presented here indicates that maize possesses a greater
403 number of putatively AM-associated *Pt* genes than, for example, rice, through expansion of
404 the *Pt13* group and presence of the *Pt14* group. It remains to be determined whether the
405 differences in transcript accumulation and loss-of-function mutant phenotype between maize,
406 on one hand, and rice and medic, on the other, stem from divergent structure of *PT* gene
407 families and variation in the partitioning of AM associated functions among the family
408 members. Indeed, the reduced colonization phenotype of the medic *pt4* mutant can be rescued
409 by nitrogen starvation contingent on the action of the ammonium transporter AMT2:3
410 (Breuillin-Sessoms et al., 2015), implicating more complex aspects of nutrient signalling
411 crosstalk in the regulation of arbuscule maintenance.

412 Although characterization of *ZmPt* transcript accumulation was consistent with a shift
413 away from direct P uptake to the AMF P uptake pathway, transcript accumulation patterns

were not clearly predictive of outcome variation among lines. The most striking difference among lines was the low level of detection of transcripts encoded by the non-AMF inducible *ZmPt8b* in the line HP301. Analysis of previously published RNA-seq transcriptome data indicated that *ZmPt8b* transcripts are among the most abundant of the *Pt* family in seedling roots, along with those encoded by *ZmPt8a* and *ZmPt2*, and the potential loss-of-function of *ZmPt8b* in HP301 may contribute to the poor performance of the line in P limiting conditions. Indeed, the observed capacity of HP301 to perform to an equivalent level as B73 when inoculated with AMF is consistent with low direct pathway PAE in HP301 being compensated through the switch to the AM P uptake pathway. There was no clear signature in the patterns of *Pt* transcript accumulation that could be related to the greater abundance of extra-radical hyphae supported by the line Oh43. Although analysis of mutants disrupted in mycorrhiza associated *PT* genes has demonstrated their requirement for establishment and maintenance of the symbiosis ( Maeda et al., 2006; Javot et al., 2007; Yang et al., 2012; Willman et al., 2013), variation in the extent of the extra-radical mycelium might be driven more directly at the level of delivery of carbohydrates to the fungus (Doidy et al., 2012a, b).

While data are consistent with shoot P accumulation being the main driver of differences in plant biomass production, significant differences were observed also in the accumulation of a number of other elements, the most variable in our PC analysis of elemental profiles being manganese (Mn), sulfur (S) and sodium (Na). Interestingly, the PC loading of Mn, S and Na was opposite to that of P, with Mn specifically showing reduced accumulation in AMF inoculated plants. In all experiments, Mn was supplied at non-limiting quantities through liquid fertilization, although availability may have been limited in the clay-sand substrate. Reduced Mn accumulation in M plants has been reported previously in maize

437 and other plants, and attributed to reduced plant production of carboxylates (e.g. Kothari et
438 al., 1991; Posta et al., 1994; Nazeri et al., 2013; Gerlach et al., 2015). Beyond this general
439 trend, we observed also variation across genotypes in Mn accumulation and an inverse
440 correlation between P and Mn accumulation that was evident in both NC and M plants.
441 Measurement of the leaf Mn concentration has been proposed as a method of distinguishing
442 different strategies of P acquisition at higher taxonomic levels (Lambers et al., 2015). Data
443 presented here indicate intraspecific variation in Mn accumulation, again potentially linked to
444 differences in root exudate production, in the presence or absence of AMF. The data also
445 clearly indicate that multiple other elements respond to variation in tissue, AMF and
446 genotype, indicating the interdependence of elemental accumulation, which may confound
447 signals derived from any single element (Baxter, 2015).

448 Attempts to identify variation in the host capacity to benefit from AM symbiosis are
449 confounded by variation in plant dependence (Sawers et al., 2008, 2010). While varieties that
450 exhibit outlying poor performance in the absence of colonization typically show significant
451 growth increase with AM inoculation, for example HP301 in this study, is does not follow
452 that such material is necessarily of interest from the point of view of crop improvement.
453 Similarly, the balance of variation in PAE and PUE in determining relative overall PE with
454 regard to other varieties will be important in terms of response variation: a line limited by
455 PAE might be projected to show greater improvement under AM symbiosis than a line
456 exhibiting comparable dependence limited by PUE. Nonetheless, the data suggest that there is
457 genetic variation in the capacity of the host plant to profit from AM symbiosis under any
458 given set of conditions. The dynamic of cost-benefit in nominally mutualistic interactions has
459 been well documented in ecological studies (e.g. Liu et al., 2012). Extrapolation to cultivated

460 systems would suggest variation impacting cost-benefit to be present, although the
461 mechanisms remain unknown. Selection of the lines Oh43 and Mo18W was based on similar
462 performance when non-inoculated, not just in terms of biomass but also shoot P, and very
463 different performance when colonized. It is interesting to note that while Oh43 supports a
464 more extensive extra-radical mycelium than the other lines evaluated, arbuscule abundance is
465 reduced, a result that is consistent with a previous report (Kaeppler et al., 2000) and perhaps
466 an indication that the interaction has been pushed towards greater fungal foraging for reduced
467 carbohydrate delivery. In contrast, Mo18W exhibited the highest abundance of intra-radical
468 fungal structures but one of the least extensive extra-radical mycelial networks. The
469 identification of such variation coupled with the availability of NAM populations for QTL
470 mapping (McMullen et al., 2009) opens up the possibility to identify markers linked to host
471 impact on the development of extra-radical mycelia with a view to implementing molecular
472 breeding strategies to target this important, but hard to evaluate, component of mycorrhiza
473 response.

474

475 **CONCLUSIONS**

476 A panel of maize inbred lines, selected to maximize genetic diversity, exhibited variation in
477 growth response when inoculated with arbuscular mycorrhizal fungi under phosphorus
478 limiting conditions. Plant growth was correlated with leaf phosphorus accumulation. The
479 accumulation of transcripts encoding members of PHT1 phosphate transporter family
480 responded variously to phosphate availability and arbuscular mycorrhizal colonization. The
481 line Oh43 that exhibited the greatest phosphorus uptake and growth response following

482 inoculation supported the most extensive extra-radical mycelium but lowest production of
483 arbuscules, suggesting that the contribution from the mycorrhiza phosphorus uptake pathway
484 is determined largely by hyphal abundance in the soil. Host genetic background impacts the
485 fungal growth strategy, *i.e.* the balance of intra-radical/extra-radical growth, with an
486 important impact on symbiotic outcome.
487

488 **MATERIALS AND METHODS**

489 **Evaluation of response to AMF in diverse maize lines**

490 A panel of 30 diverse maize lines, comprising the lines B73 W22, W64A, Pa36 and the 26
491 parents of the maize nested association mapping population (McMullen et al., 2009) , was
492 evaluated in one litre pots, under conditions of low phosphorus availability, with or without
493 inoculation with *Funneliformis mosseae* (isolate number 12, European
494 Bank of Glomales, http://www.kent.ac.uk/bio/beg/), as previously described (Sawers et al.,
495 2010) . At 8 weeks after emergence, the aerial part of the plant was harvested, dried and
496 weighed. Six experiments (A-F) were conducted in the greenhouse facility at the University
497 of Lausanne, Switzerland, during the period 2007 - 2010. Each experiment consisted of 3
498 complete replicates, with the exception of experiment D which consisted of 5 replicates.
499 Shoot dry weight data was analyzed without further transformation for clarity. Systematic
500 variation among experiments was eliminated using linear estimation. The experiment effect
501 was estimated separately for non-inoculated and inoculated plants. Mycorrhiza response was
502 estimated for each genotyping by calculation of a *t*-interval for the difference of inoculated
503 and non-inoculated means. All analysis was performed using R statistics (www.r-project.org).
504 See supplemental material for raw data, full analysis and code used to generate figures.

505

506 **Determination of elemental concentration by ICP-MS analysis**

507 Tissue samples were weighed then digested in 2.5mL concentrated nitric acid (AR Select
508 Grade, VWR) with internal standard added (20ppb In, BDH Aristar Plus). Sample digestion
509 and dilution was carried out as described in Ziegler et al., 2013. Elemental concentrations of
510 B, Na, Mg, Al, P, S, K, Ca, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Mo, and Cd were measured
511 using an Elan 6000 DRC-e mass spectrometer (Perkin-Elmer SCIEX) connected to a PFA
512 microflow nebulizer (Elemental Scientific) and Apex HF desolvator (Elemental Scientific)
513 using the procedure described in Ziegler et al. To correct for machine drift both during a
514 single run and between runs, a control solution is run every tenth sample. All analysis was
515 performed using R statistics (www.r-project.org). See supplemental material for raw data, full
516 analysis and code used to generate figures.

517 **Characterization of phosphorus uptake**

518 Six maize lines with different low P tolerance were selected and grown in compartmented 2,4
519 L PVC tubes in accordance with (Smith et al., 2003). The growth medium contained 7.9 mg
520 0.5M bicarbonate-extractable P $kg^{-1}$ (Olsen et al., 1954), was a 1:1 (w:w) mixture of sand
521 and irradiated soil (10 kGy, 10 MeV electron beam) and had basal nutrients added (Pearson
522 and Jakobsen, 1993). The root plus hyphal compartment (RHC) contained 2750 g growth
523 medium and the hyphal compartment (HC) was a small plastic vial placed in the middle of
524 the RHC. The HC contained 55 g of $^{33}P$ labelled growth medium (5 kBq $g^{-1}$) and lined with a
525 25 µm nylon mesh at both ends to prevent root in-growth. Seven weeks later, bicarbonate
526 extracts had a specific activity (SA = $^{33}P/^{31}P$) of 144.7 kBq $mg^{-1}$ P. Each maize line was
527 grown in 8 replicate pots in half of which 140 g dry soil-root inoculum of *Rhizophagus*

528 *irregularis* BEG87 was thoroughly mixed into the growth medium. Filtered BEG87 inoculum
529 leachings were added to all pots as an attempt to establish the same soil microbial community
530 (Pearson and Jakobsen, 1993) . Two pre-germinated seeds were planted in each pot and
531 thinned to one at the two leaf stage. Plants were maintained under controlled conditions (12
532 hour day length at 500 µmol $m^{-2}$ $sec^{-1}$, 28/20°C day/night and 60 % relative humidity,) and
533 watered daily by weight to 70% of the water holding capacity. In addition to the initial basal
534 nutrient dressing, supplemental N ($NH_4NO_3$), Mg and S ($MgSO_4^{2-}$) was added periodically to
535 additionally provide 375 mg N, 15 mg Mg and 20 mg S per pot. Shoots were harvested at
536 growth stage 51 (BBCH scale; tassel emergence at the top of the stem), oven dried to
537 constant weight at 70°C and dry weights were recorded. Roots system was carefully washed
538 clean using a pressurized water jet and a fine mesh to collect fine root pieces. Roots were
539 blotted dry and total fresh weight (FW) was recorded. Subsamples were taken for root
540 length/colonization measurement (1.5g FW, stored in 50% EtOH) and RNA extraction (1g,
541 flash-frozen in liquid nitrogen). Dried shoot and root samples were oxidized in a 4:1 mixture
542 (v:v) of 65% nitric:70% perchloric acids, and total P was determined by the molybdate blue
543 method using AutoAnalyzer 3 (Bran+Luebbe, Norderstedt, Germany). The $^{33}P$ in shoot tissue
544 was determined in the same digests in a Packard TR 1900 liquid scintillation counter
545 (PerkinElmer, Waltham, MA, USA). Specific activities of $^{33}P$ in shoots and in bicarbonate
546 extracts of HC soil were used to estimate the relative contribution of the AM pathway to total
547 shoot P uptake as described in Smith et al. (2004). Root length was measured by image
548 analysis using the Win-Rhizo software (Win-Rhizo version 2009b, Regent Instruments,
549 Canada) and a scanner (Epson (ModelJ1221A), Seiko Epson Corp. Japan). Images were
550 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 extent of any AM fungi structures (hyphae, arbuscules or vesicles) or arbuscules specifically was evaluated microscopically as percentage of root length using the grid-line intersect approach (Newman, 1966) after clearing and staining (Kormanik and 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 (www.r-project.org). See supplemental material for raw data, full analysis and code used to generate figures.

**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

574 GGDYPLSATIxSE motif and consequently there were not considered to be authentic PHT1
575 proteins. The final list of 13 maize PHT1 encoding gene models is presented in Table 1.

576 **Phylogenetic analyses**

577 Phylogenetic analyses were performed using the MEGA software, version 6.06 . Protein
578 sequences were aligned using MUSCLE and the resulting alignment used to construct a
579 maximum likelihood tree with 1000 bootstrap replicates. Fig. 8 was constructed using
580 predicted protein sequences corresponding to 13 candidate maize PTs, together with the
581 previously reported 13 rice proteins (Paszkowski et al., 2002) and well-characterized
582 mycorrhiza-associated proteins from trefoil (LjPT3; UniProt ID: Q1T6Z8), medic (MtPt4;
583 UniProt ID: Q8GSG4), tomato (LePT4; UniProt ID: Q563I3), potato (StPT3; UniProt ID:
584 Q8W4W9 and StPT4 UniProt ID: Q5ICC1), barley (HvPT8; UniProt ID: Q6Y3A2) and
585 wheat (TaPTmyc; UniProt ID: Q5CC72). Fig. S3 was constructed using predicted protein
586 sequences corresponding to 13 candidate maize PTs, together with the previously reported 11
587 sorghum proteins (Walder et al., 2015), 12 foxtail millet proteins (Ceasar et al., 2014) and 13
588 rice proteins (Paszkowski et al., 2002) .

589

590 **Tissue- and stage-specific *ZmPt* expression**

591 Normalized expression data for 13 maize PHT1-coding genes were retrieved from qTeller
592 (http://qteller.com/qteller3/) using two sources profiling seedling tissues (Wang et al., 2009;
593 Li et al., 2010) and one source profiling reproductive tissues (Davidson et al., 2011). Data
594 was selected for seedling root, seedling shoot, developing leaf, mature leaf (vegetative
595 tissues); and tassel, developing ear, seed 5 days-after-pollination (DAP), seed 10 DAP,
596 embryo 25 DAP and endosperm 25 DAP (reproductive tissues). Data was presented either as

absolute ($log_{10}$ counts) or relative expression across tissue types on a per gene basis. Relative values were calculated as standardized Z-scores obtained by dividing deviations from the gene mean by the gene standard deviation.

**Analysis of *ZmPt* expression in response to inoculation with AMF**

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 (Guimil et al., 2005). In total 6 phosphate transporters were analysed together with an AM specific marker gene *ZmAm3*, ortholog of *OsAM3* (Gutjahr et al., 2008; Table 5) and a *Rhizophagus irregularis* elongation factor gene (Sokolski et al., 2010). Statistical analysis was performed using R statistics (www.r-project.org). See supplemental material for raw data, full analysis and code used to generate figures.

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620

## 621 AUTHOR CONTRIBUTION

622 Evaluation of diversity panel: MM, MNG, BW, RS. Ionomic analysis: IB. Physiological
623 characterization of P uptake by six lines**:** SS, MG. Characterization of maize PHT1 family:
624 EG-M, RCM. Expression analysis: CQ. Statistical analysis: JG,RS, CQ, SS. Experimental
625 design and data interpretation IJ, UP, RS. All authors contributed to writing of the manuscript.

## 626 TABLES

627 Table 1: Mycorrhiza response in diverse maize lines

| Genotype | *NC* (g) | *M* (g) | *MR* (g) | *MR* Lower (g)[a] | *MR* Upper (g)[a] |
|---|---|---|---|---|---|
| Mo18W | 0.94 | 1.66 | 0.72 | 0.44 | 1.00 |
| Oh7b | 1.05 | 1.81 | 0.76 | 0.39 | 1.13 |
| Tzi8 | 1.48 | 2.24 | 0.76 | 0.38 | 1.15 |
| W22 | 1.15 | 1.93 | 0.78 | 0.43 | 1.13 |
| NC358 | 1.22 | 2.03 | 0.80 | 0.42 | 1.19 |
| Ky21 | 1.23 | 2.08 | 0.84 | 0.49 | 1.19 |
| Ki3 | 0.94 | 1.79 | 0.85 | 0.53 | 1.16 |

| | | | | | |
|---|---|---|---|---|---|
| CML247 | 1.08 | 1.98 | 0.90 | 0.62 | 1.18 |
| Tx303 | 0.70 | 1.61 | 0.90 | 0.46 | 1.34 |
| M37W | 0.94 | 1.91 | 0.97 | 0.66 | 1.27 |
| CML228 | 1.38 | 2.35 | 0.97 | 0.38 | 1.57 |
| CML277 | 0.93 | 1.96 | 1.03 | 0.60 | 1.45 |
| B73 | 0.78 | 1.82 | 1.05 | 0.78 | 1.31 |
| IL14H | 0.96 | 2.03 | 1.07 | 0.80 | 1.34 |
| Ki11 | 1.02 | 2.10 | 1.08 | 0.75 | 1.42 |
| CML52 | 0.81 | 1.90 | 1.10 | 0.70 | 1.49 |
| CML103 | 1.37 | 2.52 | 1.15 | 0.87 | 1.43 |
| Mo17 | 1.53 | 2.70 | 1.17 | 0.70 | 1.64 |
| CML333 | 1.19 | 2.40 | 1.20 | 0.80 | 1.61 |
| Ms71 | 1.40 | 2.62 | 1.22 | 0.71 | 1.73 |
| M162W | 1.00 | 2.23 | 1.23 | 0.79 | 1.67 |
| Pa36 | 1.67 | 2.91 | 1.23 | 0.71 | 1.76 |
| P39GB | 0.87 | 2.11 | 1.23 | 0.86 | 1.61 |
| B97 | 0.74 | 2.04 | 1.31 | 0.80 | 1.81 |

| | | | | | |
|---|---|---|---|---|---|
| NC350 | 0.86 | 2.17 | 1.31 | 0.89 | 1.73 |
| A188 | 0.98 | 2.30 | 1.32 | 0.91 | 1.73 |
| W64 | 1.02 | 2.34 | 1.32 | 1.04 | 1.61 |
| HP301 | 0.44 | 1.82 | 1.37 | 0.91 | 1.84 |
| CML322 | 1.13 | 2.59 | 1.47 | 0.80 | 2.14 |
| Oh43 | 0.96 | 2.81 | 1.85 | 1.31 | 2.38 |

628 [a]Upper and lower refer to the boundaries of a 95% t-interval for the difference M-NC

629

630 Table 2: Element accumulation in roots and leaves of non-inoculated and inoculated plants

| | root (ppm, +/- SE) | | leaf (ppm, +/- SE) | |
|---|---|---|---|---|
| ion | NC | M | NC | M |
| B11 | -66 +/-1 | -67 +/-1 | -49 +/-1.3 | -46 +/-1.4 |
| Na23 | 1400 +/-58 | 1900 +/-66 | 260 +/-21 | 330 +/-26 |
| Mg25 | 3200 +/-67 | 3100 +/-66 | 3200 +/-110 | 3000 +/-78 |
| Al27 | 1500 +/-80 | 1300 +/-59 | 36 +/-1.6 | 48 +/-2.6 |
| P31 | 300 +/-11 | 340 +/-12 | 390 +/-12 | 450 +/-13 |
| S34 | 5500 +/-150 | 6400 +/-150 | 2100 +/-38 | 2200 +/-31 |
| K39 | 21000 +/-540 | 22000 +/-500 | 28000 +/-920 | 26000 +/-780 |
| Ca43 | 6300 +/-230 | 7000 +/-310 | 5500 +/-250 | 5100 +/-200 |
| Mn55 | 240 +/-12 | 140 +/-4.8 | 82 +/-4.5 | 64 +/-2.8 |

| | | | | |
|---|---|---|---|---|
| Fe57 | 3300 +/-190 | 3000 +/-130 | 110 +/-3.7 | 140 +/-5 |
| Co59 | 3.9 +/-0.097 | 2.7 +/-0.076 | 0.085 +/-0.0041 | 0.091 +/-0.0039 |
| Ni60 | 8.8 +/-0.22 | 7.5 +/-0.2 | 0.58 +/-0.025 | 0.71 +/-0.037 |
| Cu65 | 24 +/-0.86 | 21 +/-0.76 | 15 +/-0.74 | 18 +/-1.2 |
| Zn66 | 85 +/-2.6 | 74 +/-2.1 | 46 +/-1.5 | 39 +/-1 |
| As75 | 2.4 +/-0.11 | 2.2 +/-0.079 | 0.35 +/-0.0077 | 0.36 +/-0.0082 |
| Se82 | 0.59 +/-0.36 | 0.48 +/-0.16 | 0.31 +/-0.11 | 0.018 +/-0.1 |
| Rb85 | 3.2 +/-0.098 | 3.3 +/-0.079 | 1.7 +/-0.052 | 1.8 +/-0.061 |
| Sr88 | 40 +/-0.99 | 43 +/-1.1 | 30 +/-1.5 | 26 +/-0.96 |
| Mo98 | 4.1 +/-0.16 | 4.1 +/-0.13 | 4.9 +/-0.25 | 4.5 +/-0.21 |
| Cd111 | 0.68 +/-0.013 | 0.56 +/-0.011 | 0.36 +/-0.0085 | 0.35 +/-0.0072 |

Table 3: Phosphorus transfer data

| Genotype | n | Root Length | | | Arbusculated Root Length | | | Shoot 33P activity | | | Mycorrhizal Pathway P | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | m | | | m | | | KBq | | | % | | |
| B73 | 4 | 519.0 | ± 11.6 | (a) | 442.8 | ± 101.3 | (a) | 59.4 | ± 5.23 | (bc) | 59.7 | ± 4.25 | (ab) |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hp301 | 4 | 282.1 | ± | 30 | (a) | 220.7 | ± | 26.3 | (a) | 29.1 | ± | 2.64 | (a) | 35.4 | ± | 2.95 | (a) |
| Mo17 | 3 | 481.5 | ± | 28 | (a) | 379.3 | ± | 29.0 | (a) | 59.2 | ± | 2.54 | (bc) | 60.4 | ± | 2.59 | (ab) |
| Mo18w | 4 | 513.9 | ± | 22 | (a) | 404.6 | ± | 6.3 | (a) | 49.1 | ± | 5.92 | (ab) | 52.8 | ± | 6.51 | (ab) |
| Oh43 | 4 | 432.1 | ± | 46 | (a) | 310.1 | ± | 49.0 | (a) | 77.4 | ± | 7.82 | (c) | 68.7 | ± | 7.66 | (b) |
| Pa36 | 4 | 532.6 | ± | 100 | (a) | 443.9 | ± | 84.3 | (a) | 59.6 | ± | 6.98 | (bc) | 52.3 | ± | 6.86 | (ab) |

635 Means +/- SE. Letter codes refer to means groups defined by Tukey HSD, $\alpha = 0.05$

636 Table 4: *Pht1* genes of maize (B73 Refgen v3)

| Annotation | Gene ID | Previous annotation | Position |
|---|---|---|---|
| *ZmPht1;2* | GRMZM2G070087 | | Chr1: 11.2Mb |
| *ZmPht1;5* | GRMZM2G045473 | *PT3*[b] | Chr2: 98.8Mb |
| *ZmPht1;6* | GRMZM2G112377 | PCO103837[a], *ZEAma;Pht1;3*[c] | Chr1: 202.5Mb |
| *ZmPht1;7* | GRMZM2G075870 | | Chr1: 8Mb |
| *ZmPht1;8a* | GRMZM2G154090 | *PT2*[c], *ZEAma;Pht1;1*[c] | Chr1: 236.7Mb |
| *ZmPht1;8b* | GRMZM2G326707 | PCO103838[a], | Chr5: 31.9Mb |

| | | | |
|---|---|---|---|
| | | *PT1*[b], *ZEAma;Pht1;2/4*[c] | |
| *ZmPht1;9* | GRMZM2G159075 | *PT4*[b] | Chr10: 100.0Mb |
| *ZmPht1;11* | GRMZM5G881088 | *ZEAma; Pht1;6*[c] | Chr8: 148.1Mb |
| *ZmPht1;13a* | GRMZM2G170208 | | Chr2: 99.2Mb |
| *ZmPht1;13b* | GRMZM2G041595 | *ZEAma;Pht1;5*[c] | Chr7: 172.0Mb |
| *ZmPht1;13c* | GRMZM2G009800 | | Chr2: 99.3Mb |
| *ZmPht1;13d* | GRMZM2G009779 | | Chr2: 99.3Mb |
| *ZmPht1;14* | GRMZM2G139639 | | Chr7: 34.9Mb |

637 [a]Gardiner et al., 2004

638 [b]Wright et al., 2005

639 [c]Nagy et al., 2006

640

641 Table 5 Primers used in this study

| Gene ID | Gene name | Primer sequence FW | Primer sequence REV |
|---|---|---|---|
| GRMZM2G070087 | *ZmPht1;2* | 5'-CTG CGC ATA CGC | 5'-ATT GAT TTG CTG |

| | | | |
|---|---|---|---|
| | | TAC GAA TA-3' | CAC ACG AG-3' |
| GRMZM2G045473 | *ZmPht1;5* | 5'-CCT GGA GGA GAT GTT CAG GA-3' | 5'-AAG ACG GTG AAC CAG TAG CC-3' |
| GRMZM2G112377 | *ZmPht1;6* | 5'-GCC TTC CGT TAC GTC ATT GT-3' | 5'-AGC ACG TCT CTG ATC CCA TC-3' |
| GRMZM2G075870 | *ZmPht1;7* | 5'-GGC GCT AGT AGC CAG GAA C-3' | 5'-CTG CTC CTT ATT GCC GAT GT-3' |
| GRMZM2G154090 | *ZmPht1;8a* | 5'-CAT TGT CAC GCT CGT CAT CT-3' | 5'-GGT GGA GTT GAA GTG GTC GT-3' |
| GRMZM2G326707 | *ZmPht1;8b* | 5'-CGT AGT ACG TGT GTG ATA GTC TGG-3' | 5'-TAT TAT CAC ACG TGG ACC TCT ACC-3' |
| GRMZM2G159075 | *ZmPht1;9* | 5'-CAC CAT CAT GTC GGA GTA CG-3' | 5'-AGT TCC AGC AGC AAG ATT CC-3' |
| GRMZM5G881088 | *ZmPht1;11* | 5'-GAT CCA GCT CAT CGG TTT CT-3' | 5'-GAG CGT GGT GTG TTT GTT CT-3' |
| GRMZM2G170208 | *ZmPht1;13a* | 5'-ACC GGC TAC CCT CAC CTA CT-3' | 5'-CTA CCT TCT TGG CGT CCT TG-3' |
| GRMZM2G041595 | *ZmPht1;13b* | 5'-ACT TTA TGG CAA CCG CAA TC-3' | 5'-GAG ATC CAT GAC GAG GGA GA-3' |

| | | | |
|---|---|---|---|
| GRMZM2G009779 | *ZmPht1;13d* | 5'-CAC TAT TTG CCT CTA ACC TAG TCG-3' | 5'-GAA TCT GGT AGA AGG AAG GTA AAC AC-3' |
| GRMZM2G139639 | *ZmPht1;14* | 5'-TCA CTG GAT TTA ATT TGT GCT CAT-3' | 5'-CGG AGA CAG GTT GTT GTT TTA GAT-3' |
| GRMZM2G135244 | ZmAM3 | 5'-ATC TGT CGT TGC GTT CCT CT-3' | 5'-GCA TCT ATC ACT GCG GGA AT-3' |
| GRMZM2G046804 | GAPDH | 5'-CTT CGG CAT TGT TGA GGG TTT G-3' | 5'-TCC TTG GCT GAG GGT CCG TC-3' |

642

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645

646 **FIGURE LEGENDS**

647 **Figure 1.** Association with mycorrhizal fungi promotes vegetative growth in diverse maize
648 lines. Distribution of shoot dry weights (SDW, g; normalized with respect to differences
649 among replicates) of 8 week-old maize plants grown with (red) or without (blue) inoculation
650 with the fungus *Funneliformis mosseae*. Data pooled across 30 diverse maize lines and
651 normalized with respect to variation between plantings. The mean SDW of non-inoculated
652 (2.16g, n = 552) and inoculated (1.05g, n = 540) groups is shown by dotted lines and the
653 mycorrhizal response (MR) indicated as the difference in means (1.1g ± 0.08g; 95% interval
654 for difference in means). Outlying samples > 4g SDW not shown; samples adjusted to < 0g
655 SDW after normalization were set to 0g.

656

657 **Figure 2.** Diverse maize lines vary in mycorrhiza response. Main panel, shoot dry weight
658 (SDW, g; normalized with respect to differences among replicates) of 30 diverse maize lines
659 grown for 8 weeks, under greenhouse conditions, with (white boxes) or without (grey boxes)
660 inoculation with the fungus *Funneliformis mosseae*. Boxes show 1st quartile, median and 3rd
661 quartile. Whiskers extend to the most extreme points within 1.5x box length; outlying values
662 beyond this range are not shown. The mean values of non-inoculated (1.05g, n=540) and
663 inoculated ( 2.16g, n=552) groups are shown by horizontal blue and red lines, respectively.
664 Upper panel, mycorrhizal response (MR, g) calculated as the difference in SDW of inoculated
665 and non-inoculated plants. Green points indicate the difference M-NC; whiskers extend to the
666 limits of a 95% confidence interval; horizontal dashed line shows the mean MR across all
667 lines of 1.1g. Lines ordered by increasing MR from left to right. Data as Figure 1.

668

669 **Figure 3.** The line Oh43 shows the greatest mycorrhizal response. Shoot dry weight (SDW, g;
670 normalized with respect to differences among replicates) reaction norms for 30 diverse maize
671 lines contrasting non-inoculated plants (NC; blue points) and plants inoculated with the
672 fungus *Funneliformis mosseae* (M; red points). Segments corresponding to the selected lines
673 Oh43 and Mo18W are highlighted in bold. Line names are shown adjacent to the
674 corresponding point unless a number of points are superimposed in which case line names are
675 off-set and listed in rank order. Data as Figure 1.
676
677 **Figure 4.** Inoculation with AMF is associated with changes in the ionome. A, Accumulation
678 of 20 ions in the roots and leaves of 8 week-old maize plants grown with (M) or without (NC)
679 inoculation with the fungus *Funneliformis mosseae*. Mean accumulation was calculated
680 across 30 diverse maize lines (NC root, n=117; M root, n=109; NC leaf, n=107; M leaf,
681 n=126), standardized (Z-score) per ion across treatments, and represented on a scale from
682 white (below average accumulation) to brown (above average accumulation). Ions which
683 accumulated differentially between NC and M ($p<0.05$, adjusted for multiple tests) in either
684 roots and/or leaves are indicated by an asterisk in the appropriate M column or columns.
685 Analysis performed on a subset of the samples shown in Figure 1. B, Principal component
686 (PC) loading (rotation) for ten AMF responsive ions (Na, Al, P, S, Mn, Fe, Co, Ni, Zn, Cd;
687 conventional colouring) in a PC analysis. Loading in the first two PCs is shown (arbitrary
688 scale), explaining 75% and 8% of the total variation, respectively. Dashed lines indicate zero
689 loading. C, Individual scores for the first two PCs (PC1; PC2) and corresponding
690 standardized accumulation of P (orange points), Mn (grey points) and S (yellow points). The

691 two clear groups defined by PC1 correspond to leaf and root samples (indicated). A number
692 of outlying individuals are omitted for clarity. Data as A.
693
694 **Figure 5.** Oh43 and Mo18W differ in elemental profile. Principal component (PC) scores for
695 the first three PCs (PC1; PC2; PC3; percentage values in parentheses indicate proportion of
696 variation explained) of a PC analysis of variation in the mean level of accumulation of 20
697 ions in the roots (squares) and leaves (circles) of 30 diverse maize lines grown with (red
698 points) or without (blue) inoculation with the fungus *Funneliformis mosseae* (subset of
699 samples from Figure 1; PC analysis as Figure 4). Dashed lines indicate zero loading. Scores
700 corresponding to the selected lines Oh43 and Mo18W are labelled and shown as larger, more
701 prominently coloured symbols. Colour bars indicate a linear fit of the relationship between P
702 accumulation and the PC shown on the adjacent axis: in both plots movement towards the
703 lower left quadrant is associated with increased P accumulation. The two clear groups defined
704 by PC1 correspond to leaf and root samples (indicated). A number of outlying individuals are
705 omitted for clarity.
706
707 **Figure 6.** Shoot growth is correlated with P uptake in six maize genotypes with contrasting
708 mycorrhizal response. A, B73 plants grown without (left) or with (right) inoculation with
709 *Rhizophagus irregularis*. B and C, main panels, shoot dry weight (SDW; g) and shoot P
710 content (Shoot P; mg) of selected maize lines grown with (M; red box) or without (NC; blue
711 box) inoculation. Boxes show 1st quartile, median and 3rd quartile. Whiskers extend to the
712 most extreme points within 1.5x box length; outlying values beyond this range are not shown.
713 B and C, upper panels, mycorrhizal response (MR; calculated as the difference of inoculated

714 (M) and non-inoculated (NC) plants. Green points indicate the difference $\mu_M$-$\mu_{NC}$; whiskers
715 extend to the limits of a 95% confidence interval; horizontal dashed line shows the mean MR
716 ($\mu_{MR}$). D, scatter plot of mean shoot P (mg) against mean SDW (g) for selected maize lines,
717 with (red) or without (blue) inoculation.
718
719 **Figure 7.** P accumulation is correlated with the abundance of extra-radical hyphae. A,
720 Percentage of total root length containing mycorrhizal structures (dark-red) and arbuscules
721 (light-red). B, Relationship between shoot P and hyphal length in the hyphae compartment of
722 mycorrhizal plants. C, Shoot content of $^{33}$P in relation to the arbusculated root length (ARL)
723 of mycorrhizal plants. Refer to Fig. 2 for a guide to the boxplots. Letters indicate significant
724 differences ($p<0.05$, Tukey HSD)
725
726 **Figure 8.** Mycorrhiza associated PHT1 proteins form distinct clades. Maximum likelihood
727 phylogeny generated from the complete set of maize (Zm) and rice (Os) PHT1 proteins and
728 selected mycorrhiza-associated PHT1 proteins from trefoil (Lj), medic (Mt), tomato (Le),
729 potato (St) and barley (Hv). Yeast PHO84 (Sc) used as outgroup. Bootstrap support values are
730 shown as percentage at the nodes.
731
732 **Figure 9.** *ZmPt* transcript accumulation responds to P availability and AM colonization.
733 Accumulation of *ZmPt* (*Pt*) transcripts quantified relative to *beta-actin* by real-time PCR and
734 represented as heatmaps. A) Accumulation of 10 *ZmPt* transcripts detected in B73 seedling
735 shoot or root tissue. Plants were grown across a range of increasing P availability at 10µM,
736 100µM, 1000µM P without inoculation with AMF (NC) and also at 100µM with inoculation

737 with *R. irregularis* at 100µM (M). Mean accumulation was determined from three biological
738 replicates, standardized (Z-score) within transcripts across experimental treatments and
739 represented on a scale from white (below average accumulation) to brown (above average
740 accumulation). Accumulation of the maize mycorrhizal marker transcript *Am3* and the *R.*
741 *irregularis* elongation factor *RiEF* is also shown. Transcripts that accumulated differentially
742 between M and NC in a given tissue (Tukey HSD, $\alpha$=0.05) are marked with an asterisk in the
743 appropriate M column. B) Accumulation of six *ZmPt* (*Pt*) transcripts in root-samples of the
744 selected lines B73, Mo17, Hp301, Pa36, Mo18W and Oh43, grown under high P (P+; 53.2
745 mg $kg^{-1}$) or low P (P-; 7.9 mg $kg^{-1}$) with (M) or without (NC) inoculation with *Rhizophagus*
746 *irregularis*. Mean transcript accumulation was determined from three biological replicates,
747 scaled independently for each gene panel from white (minimum) to brown (maximum)
748 accumulation. Accumulation of *Am3* and *RiEF* is also shown.

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**Figure 1.** Association with mycorrhizal fungi promotes vegetative growth in diverse maize lines. Distribution of shoot dry weights (SDW, g; normalized with respect to differences among replicates) of 8 week-old maize plants grown with (red) or without (blue) inoculation with the fungus *Funneliformis mosseae*. Data pooled across 30 diverse maize lines and normalized with respect to variation between plantings. The mean SDW of non-inoculated (2.16g, n = 552) and inoculated (1.05g, n = 540) groups is shown by dotted lines and the mycorrhizal response (MR) indicated as the difference in means (1.1g ± 0.08g; 95% interval for difference in means). Outlying samples > 4g SDW not shown; samples adjusted to < 0g SDW after normalization were set to 0g.

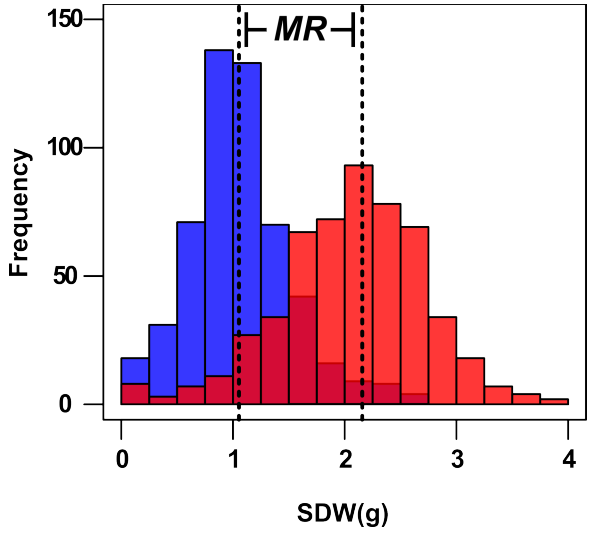


**Figure 2.** Diverse maize lines vary in mycorrhiza response. Main panel, shoot dry weight (SDW, g; normalized with respect to differences among replicates) of 30 diverse maize lines grown for 8 weeks, under greenhouse conditions, with (white boxes) or without (grey boxes) inoculation with the fungus *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 non-inoculated (1.05g, n=540) and inoculated ( 2.16g, n=552) groups are shown by horizontal blue and red lines, respectively. Upper panel, mycorrhizal response (MR, g) calculated as the difference in SDW of inoculated and non-inoculated plants. Green points indicate the difference M-NC; whiskers extend to the limits of a 95% confidence interval; horizontal dashed line shows the mean MR across all lines of 1.1g. Lines ordered by increasing MR from left to right. Data as Figure 1.

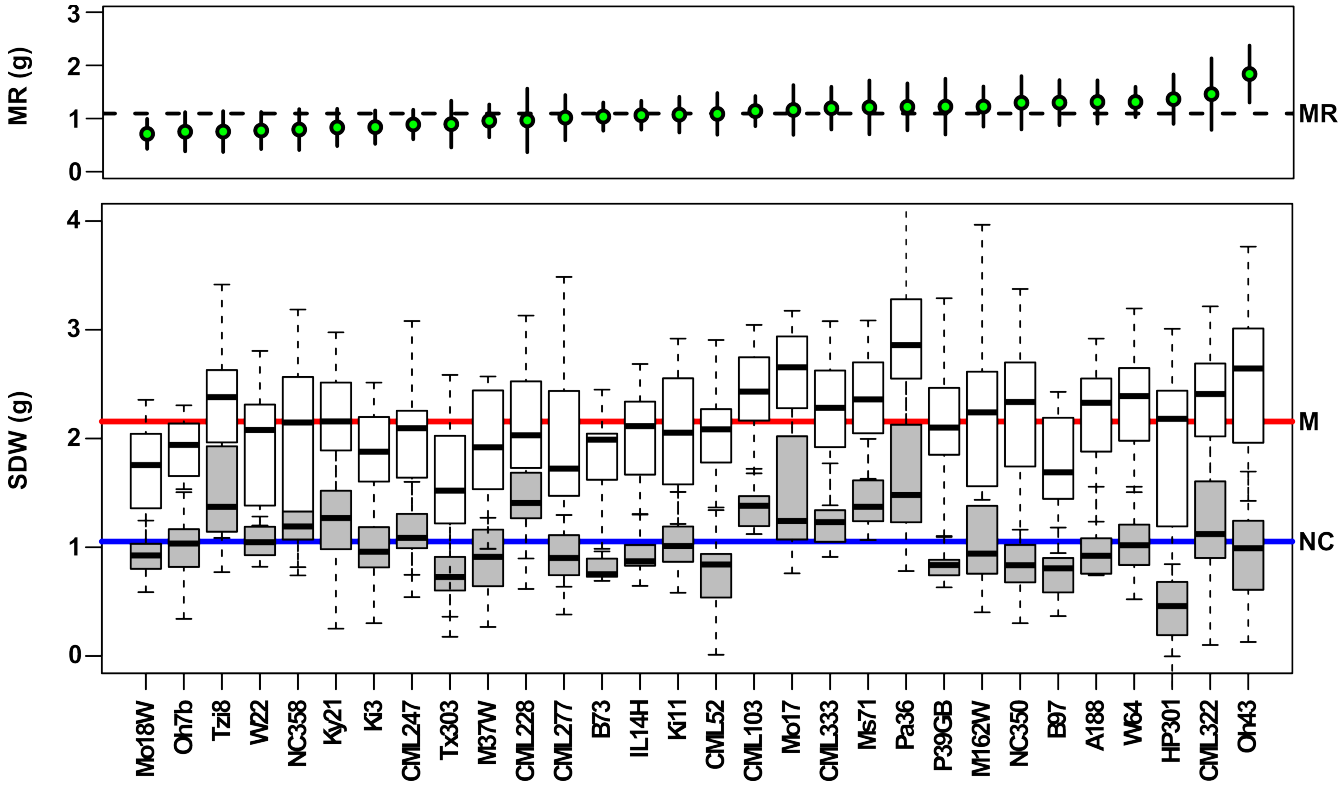


**Figure 3.** The line Oh43 shows the greatest mycorrhizal response. Shoot dry weight (SDW, g; normalized with respect to differences among replicates) reaction norms for 30 diverse maize lines contrasting non-inoculated plants (NC; blue points) and plants inoculated with the fungus *Funneliformis mosseae* (M; red points). Segments corresponding to the selected lines Oh43 and Mo18W are highlighted in bold. Line names are shown adjacent to the corresponding point unless a number of points are superimposed in which case line names are off-set and listed in rank order. Data as Figure 1.

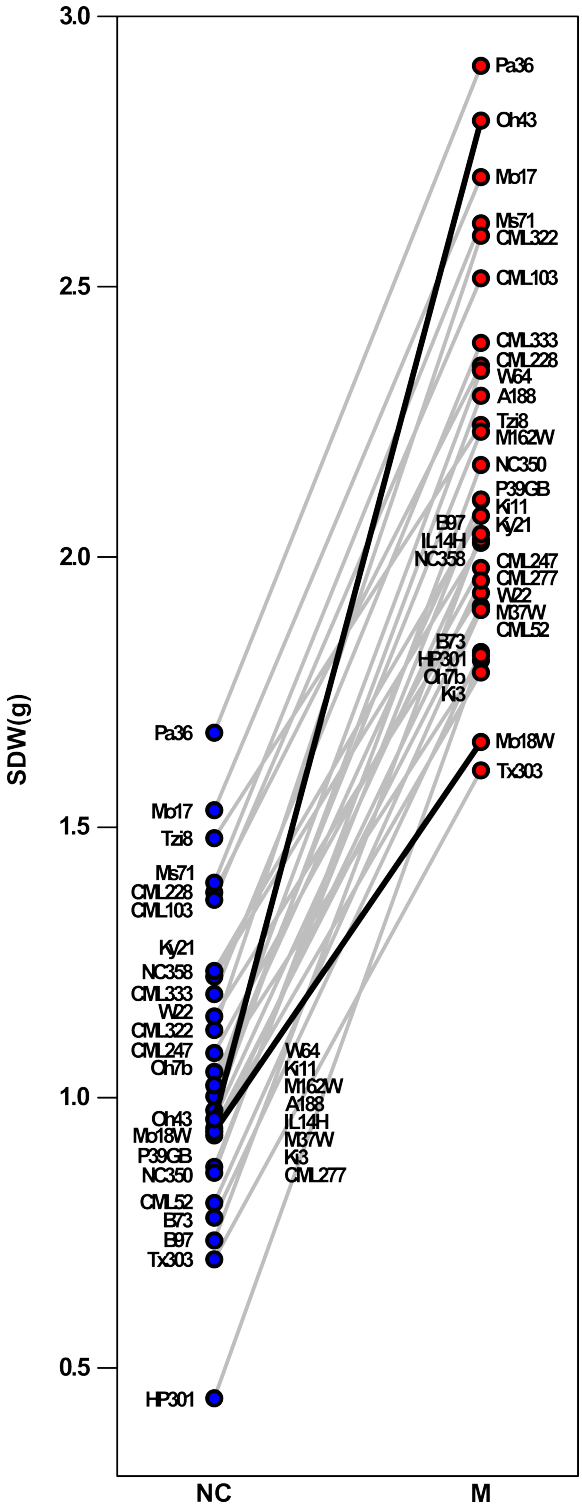


**Figure 4.** Inoculation with AMF is associated with changes in the ionome. A, Accumulation of 20 ions in the roots and leaves of 8 week-old maize plants grown with (M) or without (NC) inoculation with the fungus *Funneliformis mosseae*. Mean accumulation was calculated across 30 diverse maize lines (NC root, n=117; M root, n=109; NC leaf, n=107; M leaf, n=126), standardized (Z-score) per ion across treatments, and represented on a scale from white (below average accumulation) to brown (above average accumulation). Ions which accumulated differentially between NC and M ($p<0.05$, adjusted for multiple tests) in either roots and/or leaves are indicated by an asterisk in the appropriate M column or columns. Analysis performed on a subset of the samples shown in Figure 1. B, Principal component (PC) loading (rotation) for ten AMF responsive ions (Na, Al, P, S, Mn, Fe, Co, Ni, Zn, Cd; conventional colouring) in a PC analysis. Loading in the first two PCs is shown (arbitrary scale), explaining 75% and 8% of the total variation, respectively. Dashed lines indicate zero loading. C, Individual scores for the first two PCs (PC1; PC2) and corresponding standardized accumulation of P (orange points), Mn (grey points) and S (yellow points). The two clear groups defined by PC1 correspond to leaf and root samples (indicated). A number of outlying individuals are omitted for clarity. Data as A.

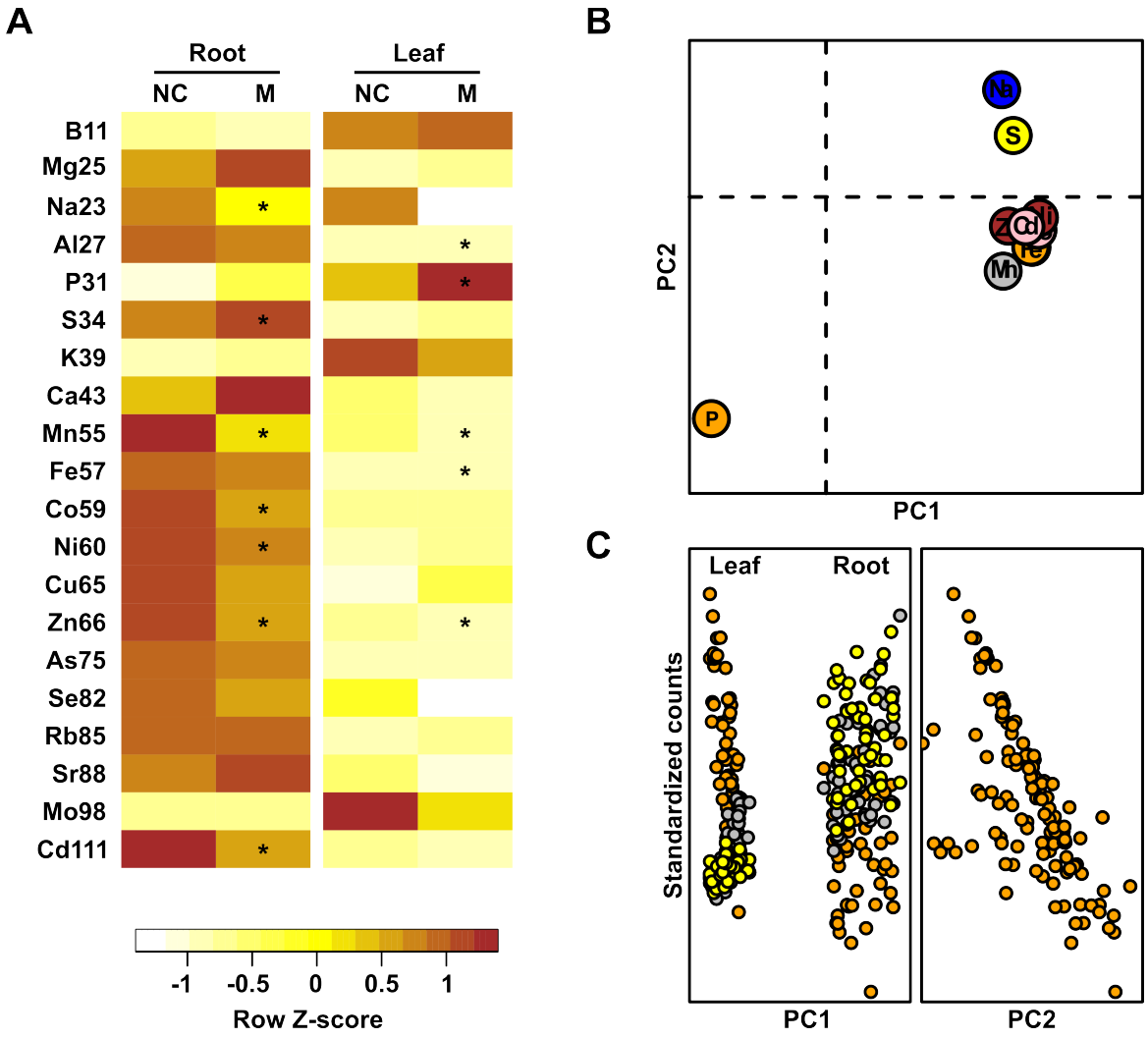
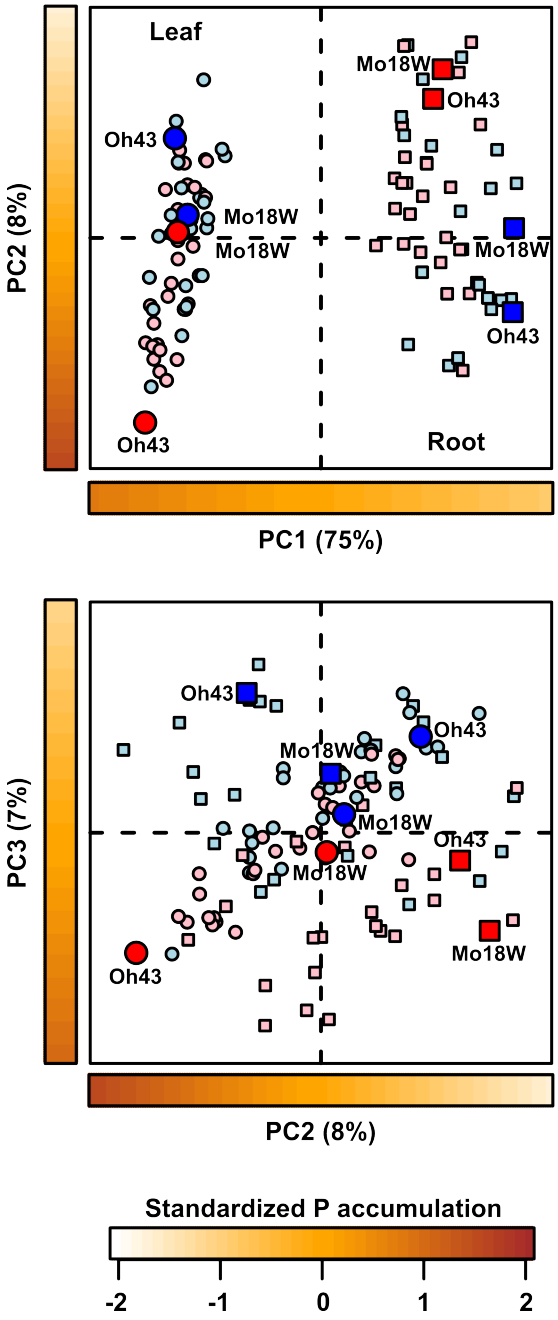


**Figure 5.** Oh43 and Mo18W differ in elemental profile. Principal component (PC) scores for the first three PCs (PC1; PC2; PC3; percentage values in parentheses indicate proportion of variation explained) of a PC analysis of variation in the mean level of accumulation of 20 ions in the roots (squares) and leaves (circles) of 30 diverse maize lines grown with (red points) or without (blue) inoculation with the fungus *Funneliformis mosseae* (subset of samples from Figure 1; PC analysis as Figure 4). Dashed lines indicate zero loading. Scores corresponding to the selected lines Oh43 and Mo18W are labelled and shown as larger, more prominently coloured symbols. Colour bars indicate a linear fit of the relationship between P accumulation and the PC shown on the adjacent axis: in both plots movement towards the lower left quadrant is associated with increased P accumulation. The two clear groups defined by PC1 correspond to leaf and root samples (indicated). A number of outlying individuals are omitted for clarity.



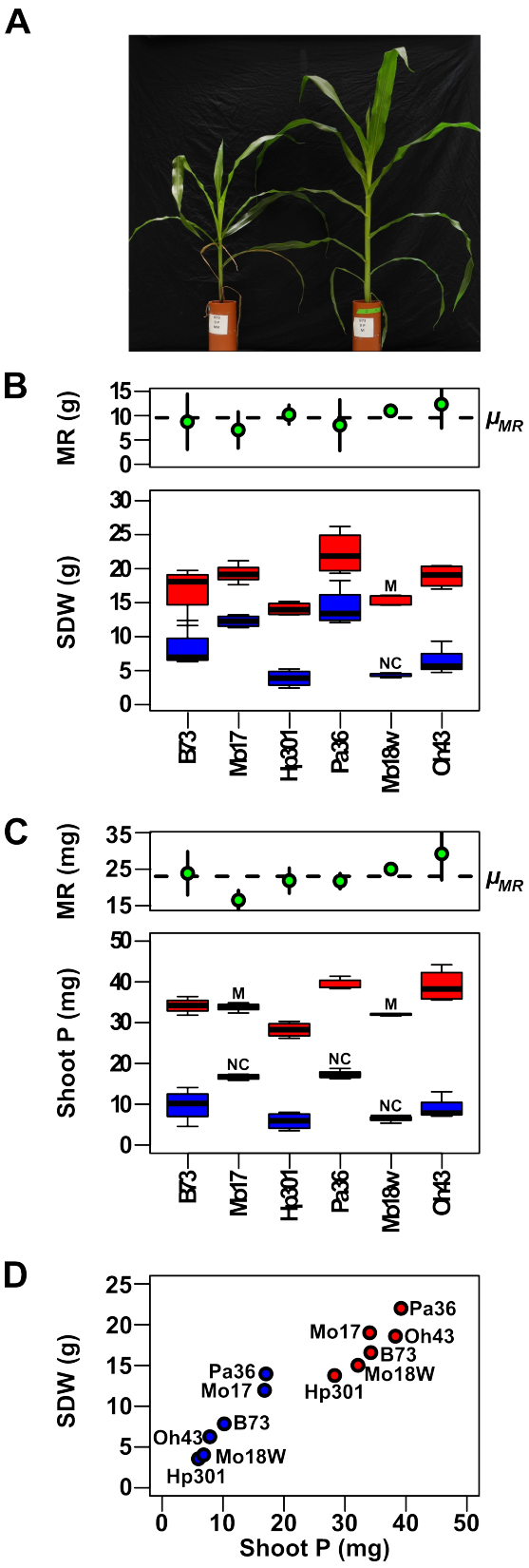



**Figure 6.** Shoot growth is correlated with P uptake in six maize genotypes with contrasting mycorrhizal response. A, B73 plants grown without (left) or with (right) inoculation with *Rhizophagus irregularis*. B and C, main panels, shoot dry weight (SDW; g) and shoot P content (Shoot P; mg) of selected maize lines grown with (M; red box) or without (NC; blue box) inoculation. 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. B and C, upper panels, mycorrhizal response (MR; calculated as the difference of inoculated (M) and non-inoculated (NC) plants. Green points indicate the difference $\mu_M$-$\mu_{NC}$; whiskers extend to the limits of a 95% confidence interval; horizontal dashed line shows the mean MR ($\mu_{MR}$). D, scatter plot of mean shoot P (mg) against mean SDW (g) for selected maize lines, with (red) or without (blue) inoculation.

**Figure 7.** P accumulation is correlated with the abundance of extra-radical hyphae. A, Percentage of total root length containing mycorrhizal structures (dark-red) and arbuscules (light-red). B, Relationship between shoot $^{33}P$ and hyphal length in the hyphae compartment of mycorrhizal plants. C, Shoot content of $^{33}P$ in relation to the arbusculated root length (ARL) of mycorrhizal plants. Refer to Fig. 2 for a guide to the boxplots. Letters indicate significant differences ($p<0.05$, Tukey HSD)

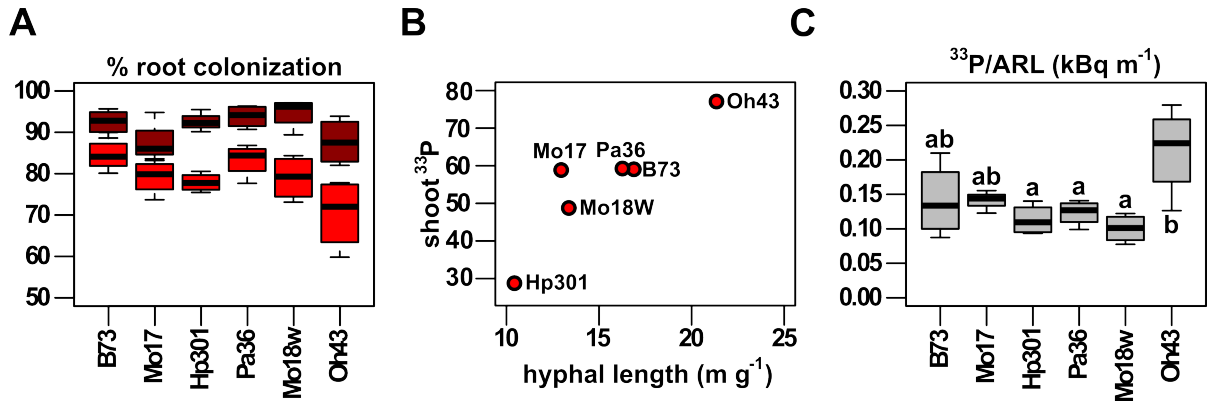


**Figure 8.** Mycorrhiza associated PHT1 proteins form distinct clades. Maximum likelihood phylogeny generated from the complete set of maize (Zm) and rice (Os) PHT1 proteins and selected mycorrhiza-associated PHT1 proteins from trefoil (Lj), medic (Mt), tomato (Le), potato (St) and barley (Hv). Yeast PHO84 (Sc) used as outgroup. Bootstrap support values are shown as percentage at the nodes.

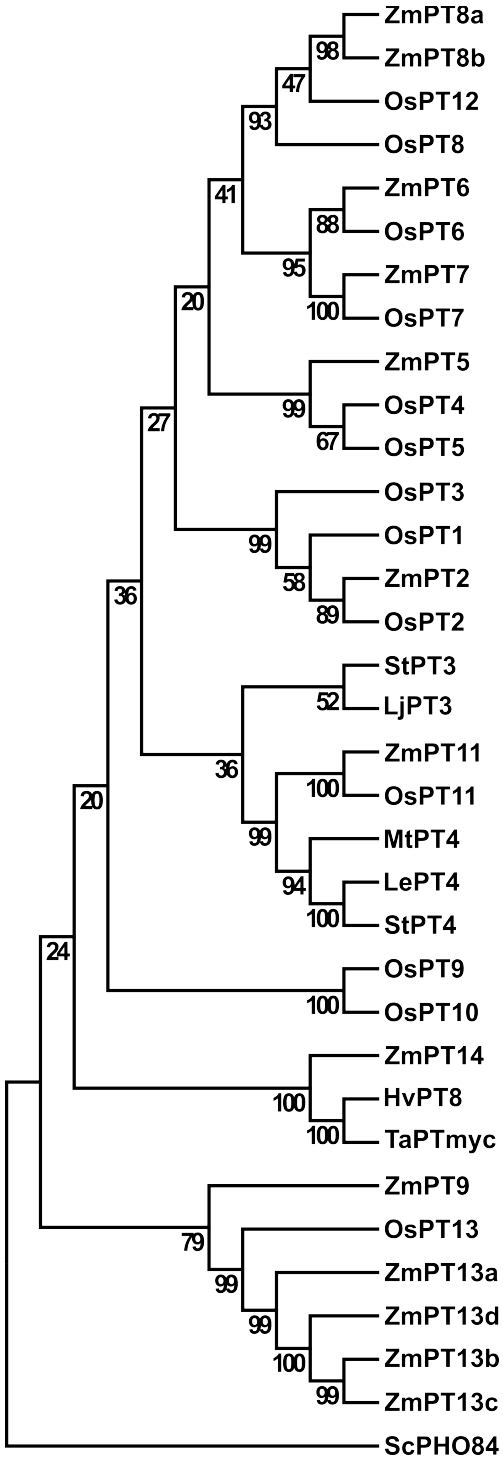
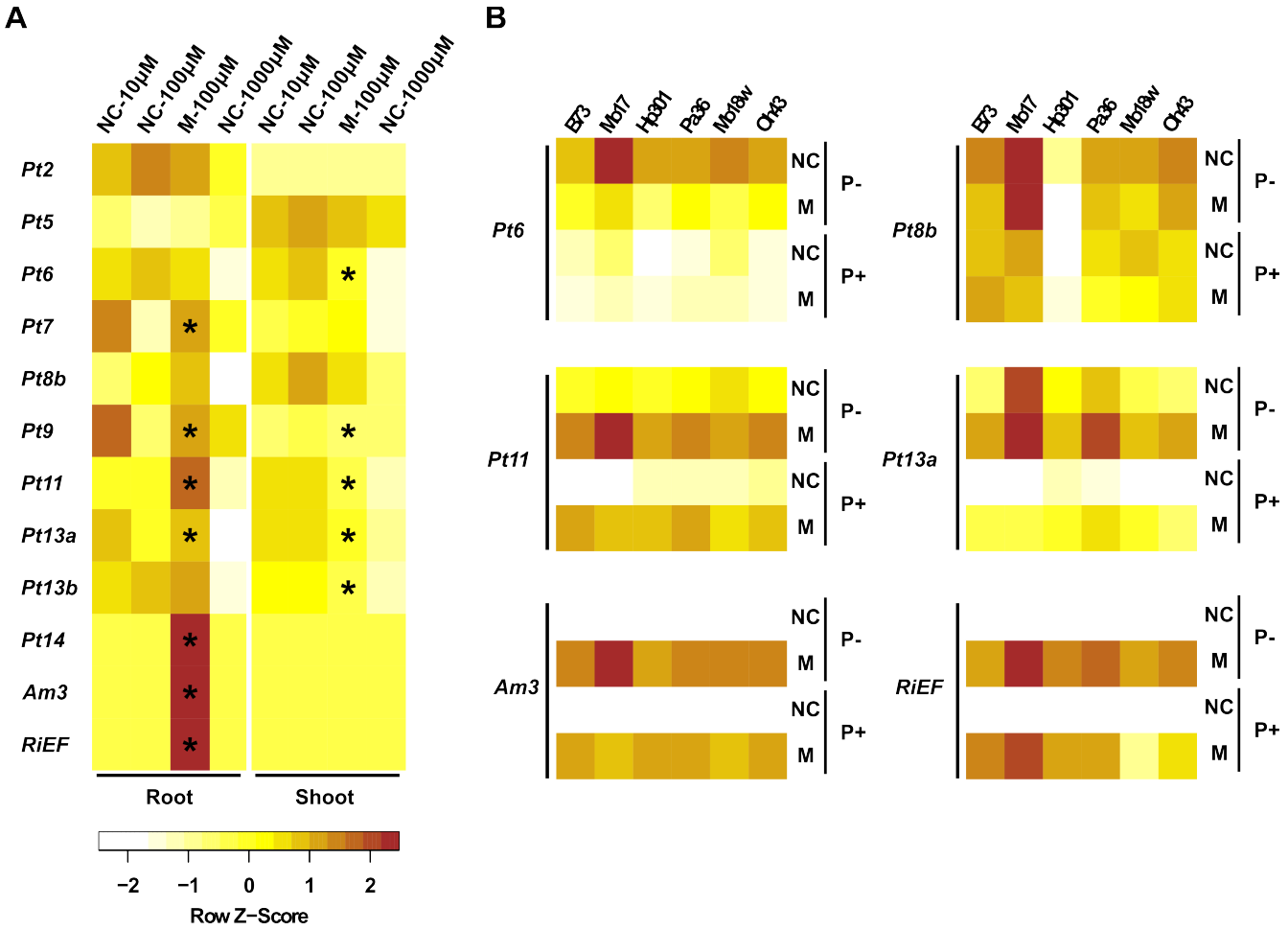


**Figure 9.** *ZmPt* transcript accumulation responds to P availability and AM colonization. Accumulation of *ZmPt* (*Pt*) transcripts quantified relative to *beta-actin* by real-time PCR and represented as heatmaps. A) Accumulation of 10 *ZmPt* transcripts detected in B73 seedling shoot or root tissue. 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 that accumulated differentially between M and NC in a given tissue (Tukey HSD, α=0.05) are marked with an asterisk in the appropriate M column. B) Accumulation of six *ZmPt* (*Pt*) transcripts in root-samples of the selected lines B73, Mo17, Hp301, Pa36, Mo18W and Oh43, grown under high P (P+; 53.2 mg kg-1) or low P (P-; 7.9 mg kg-1) with (M) or without (NC) inoculation with *Rhizophagus irregularis*. Mean transcript accumulation was determined from three biological replicates, scaled independently for each gene panel from white (minimum) to brown (maximum) accumulation. Accumulation of *Am3* and *RiEF* is also shown.



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