Potential of Acid Phosphatase Activity and Mycorrhizal Colonization to Reduce Phosphorus Fertilization in Maize Cultivated in Brazil

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Research Article

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Abstract

Purpose: It is urgent to mitigate the environmental impacts resulting from agriculture, especially in highly biodiverse and threatened areas, as the Brazilian Cerrado. We aim to investigate whether phosphatase activity and mycorrhizal colonization are alternative plant strategies for nutrient acquisition in maize cultivated under fertilized and unfertilized conditions in Brazil, potentially contributing to reduce the use of phosphate fertilizers needed for production.

Methods: Three experiments were performed: the first was conducted in a glasshouse, with 17 experimental pure maize lineages and two phosphorus treatments; the second in the field, with 3 pure maize lineages and two treatments, one without fertilization and another with NPK fertilization; and the third was also carried out in the field, with 13 simple commercial hybrids, grown either under NK or under NPK treatment. Soil and plant variables were measured and tested for the response to fertilization, differences amongst genotypes and response to phosphatase activity and mycorrhizal colonization.

Results: We detected a positive effect of mycorrhizal colonization upon growth in pure maize lineages. The activity of acid phosphatase was modulated by the availability of phosphorus and nitrogen in the soil, and promoted grain filling of commercial hybrids in soils with low phosphorus availability.

Conclusions: These results demonstrate, for the first time, that it is possible to select genotypes that are more adapted to low soil phosphorus availability aiming at organic production, or to use genotypes that have high phosphatase activity under phosphorus fertilization to reduce the amount of added phosphorus needed for maize production in Brazil.

1. Introduction

To better conciliate socioeconomic development with nature protection, two main sustainability goals for 2030 are the mitigation of the environmental impacts caused by agriculture and the increase of agricultural productivity in regions of low technology and low soil nutrient availability (United Nations 2015). Sustainable production in agriculture has received attention in the last years due to an urgent need to mitigate environmental degradation caused by environmentally unfriendly agricultural practices, being urgent the increase of productivity in organic systems and the reduction on use of artificial fertilizers (Kirchmann and Thorvaldsson 2000).

Phosphorus (P) is an essential element for plant growth because it composes the adenosine triphosphate (ATP), acts on cell division, reproduction and on crucial metabolic activities as photosynthesis and respiration. This nutrient widely limits plant growth and productivity in agricultural systems, especially in tropical areas (Hou et al. 2020). The wide use of phosphate fertilizers, however, can be environmentally harmful, causing soil and water eutrophication and culminating in biodiversity losses in tropical areas. In addition, phosphate fertilizers are costly, have finite sources and are potentially ineffective due to soil P immobilization.

The global dependence on the use of P fertilizers for sustaining crop production is a matter of growing concern due to the lack of accurate information about the world's remaining clean P reserves and to the predictions about their exhaustion until the end of this century, which may result in exponential price increases, and even set it as an instrument of food security (Gilbert 2009; Mew 2016). It is estimated that Brazil is responsible for nineteen percent of the world phosphorus consumption, importing sixty percent of those and placing the country as the second major importer of P in the world (Filho 2014). The Brazilian Cerrado is a biome that occupies an area of 2 million km^2 and is considered as a biodiversity hotspot (Myers et al. 2000) because it contains the most plant diverse savannas in the world with high level of endemism (Klink and Machado 2005) under permanent threat due to the expansion of conventional intensive farming (Lima et al. 2019) lacking proper environmental governance. In this area, phosphorus additions upon natural plant communities promote ecosystem degradation directly due to biodiversity losses (Bustamante et al. 2012; Lannes et al. 2016) and indirectly due to the promotion of exotic plant invasions (Lannes et al. 2020a). These facts highlight the importance of reducing phosphorus inputs in the Cerrado to lessen its harmful effects upon biodiversity and to conserve global P reserves, and one way to reach this goal is by improving the P use efficiency of major crops grown in the region.

The main P form used by plants in the Cerrado region is the dihydrogen phosphate ($H_2PO_4^-$) due to the low soil pH. In these soils, P generally gets immobilized due to the fixation to iron, aluminium, calcium and clayey minerals (Vance et al. 2003). The Cerrado, as a rich reservoir of genetic resources, has wild plants with several adaptive strategies to overcome low P availability (Lambers et al. 2020), but cultivated plants also present a wide genotypic variation on strategies for P acquisition (Cong et al. 2020). Plant strategies in P-deficient soils involve structural root modifications, mycorrhizal associations, formation of cluster roots and the secretion of the acid phosphatase enzyme (PME, hereafter) (Lambers et al. 2006; Balemi and Negisho 2012). This enzyme is released by plants and microorganisms and its activity is responsible for the active mobilization of organic P, cleaving phosphate from phosphatomonoesters and phosphatodiesters, having optimum activity under acid conditions and increasing overall plant P absorption (Gonzalez-Munoz et al. 2015).

Organic and inorganic P, as well as inorganic nitrogen (N) availability are the most influential factors determining PME activity. High levels of inorganic P inhibit the activity, whereas high levels of organic P (substrate) and inorganic N stimulate it (Olde Venterink and Güsewell 2010; Margalef et al. 2017). Soil pH also influences PME activity, acting on the levels of synthesis, release and stabilization of the enzyme. Other factors that negatively influence PME activity are fires, as well as low soil moisture and the presence of lead and other heavy metals (Adetunji et al. 2017). Considering that Cerrado soils are deficient in inorganic but rich in organic P (Lannes 2020a), to understand the levels of PME activity by crop plants can be an excellent way for optimizing the organic management of the soil, resulting in better soil conservation and agricultural sustainability, since a considerable part of the assimilated P is produced from organic P mineralization through PME activity (Adetunji et al. 2017).

Arbuscular mycorrhizae fungi (AMF) generally colonize the root system of most plants, contributing to nutrient uptake especially when soil P contents are low. They favour growth of root hairs and have extensive hyphae network, which allows plants to explore larger soil volumes. These fungi can contribute to more sustainable farming but the efforts in this sense are scarce, therefore it is necessary to develop technology for such aim, demanding more studies of this kind (Rillig et al. 2016).

Maize is a major crop worldwide and it has a high production potential in Brazil, reaching 10 tons per hectare of grains in experimental conditions and when cultivated adequately. However, lower productivities are generally reached, around 3.5 tons per hectare (Carvalho et al. 2004). Due to the high P adsorption in Brazilian soils, the low efficiency of P use in maize (around 25%) causes recommended fertilizing dosages to be extremely high. Besides, maize has high P demand (Dhillon et al. 2017) because of the intense and short cycle, which requires higher P levels in soil solution than perennial crops.

A substantial genetic variability is found in maize, which allows it to be cultivated under several environmental conditions with specific adaptations, and crop breeding can contribute to productivity enhancement by developing new varieties and hybrids that are highly adapted to the environment. Breeding for enhancing P absorption in maize depends on the identification of a trait that potentially influences P acquisition and use and on identification or generation of genetic variation within a germoplasm source (Vance et al. 2003). The release of PME in maize is a quantitative character, as shown by Gonzalez-Munoz et al. (2015). Chen et al. (2008) estimated the broad-sense heritability of 52.7% for PME activity and of 90.9% for P absorption efficiency in maize, confirming that genetics in an important factor for PME activity of this crop species.

Studies point out that, amongst a wide range of acid phosphatases codified by plants, the purple acid phosphatases (PAPs) have a significant role on P foraging and recycling (Tran et al. 2010). In maize, Gonzalez-Munoz et al. (2015) identified 33 genes related to PAPs, showing that the expression of this character is quantitative and confirming that there are important functional variations within maize germoplasm. The authors also reported that accumulation of transcripts of the gene ZmPAP26 was not different under high- and low-P availabilities, differently to other 19 PAPs genes that had higher PME activity under lower P availability. This suggests that the PAPs genes in maize have several functions in post-transcriptional regulation and possibly the functional divergence in higher than known so far.

Mycorrhiza fungi are important for maize because this crop presents high growth rates and high nutrient demand, therefore the plant-fungi interaction is positive for growth, grain production and P uptake (Cozzolino et al. 2013). However, this increase resulting from the symbiosis varies within genotypes in terms of aerial and root production (Campos et al. 2010). Maize growth in response to mycorrhizal colonization is generally positive, but it depends on the balance between N and P in the soil and is more important for P than for N uptake (López-Carmona et al. 2019).

Due to the scarce information found in the literature in relation to PME activity and mycorrhizal colonization in pure lineages and commercial hybrids of maize, there is a potential to explore these characteristics aiming at improving crop growth towards more sustainable maize production. A better

understanding of these factors can, therefore, contribute to the reduction of phosphate fertilizers needed in maize production and help genetic enhancement through breeding in search of genotypes that are better adapted to more sustainable agricultural systems. Based on the abovementioned premises, the main hypotheses of this research are (1) PME activity and mycorrhizal colonization rates differ in various genotypes cultivated in the Cerrado, (2) PME activity and mycorrhizal colonization rates are modulated by inorganic P addition, with higher values found under natural cultivation than under traditional P addition, and (3) Maize growth and productivity are affected by PME activity and mycorrhizal colonization rates.

We aim at verifying the performance of various maize genotypes (pure experimental lineages and simple commercial hybrids) in relation to PME activity and mycorrhizal colonization rates under different soil inorganic P availabilities (Fig. 1). Specifically, we aim at investigating whether (1) different genotypes grown in the Cerrado have different PME activities, (2) phenotypic plasticity for PME activity in relation to fertilization occurs, (3) mycorrhizal colonization is influenced by phosphorus addition in maize commercial hybrids, and (4) PME activity and mycorrhizal colonization affect growth and productivity under natural and P fertilized conditions.

2. Methods

2.1. Glasshouse study

A glasshouse study was conducted from August to October 2018 in Ilha Solteira, São Paulo State (20°25’04.77”S 51°20’30.65”O, 375 m elevation). We used 3.5 L pots filled with 2 mm-sieved Cerrado soil (dystrophic Red Latosol – Oxisol) (Santos et al. 2018). From seeds, seventeen genotypes were cultivated under each of two treatments (control – only distilled water added, or P fertilization – 0.09 mg sodium phosphate (Na_2HPO_4), equivalent to 200 mg $P.kg^{-1}$ soil, as suggested by Novais et al. (1985)), in three replicates, in a total of 102 pots. These pure lineages were chosen because they are experimental genotypes developed at São Paulo State University (Unesp Ilha Solteira) and are potential candidates for a future breeding program (Appendix 1).

In the V9 growth stage of the plants (Ritchie et al. 2003) we evaluated PME activity, mycorrhizal colonization rates, plant height, number of leaves, stem diameter, water content, aerial dry biomass, root dry biomass and total dry biomass. The measurements were performed during this plant stage because it represents the end of the plant vegetative stage and the start of the most P-demanding stage of the crop.

Root phosphatase activity was measured using 100 mg fresh roots in 5 ml p-NPP (*para*-nitrophenylphosphate). Root samples were taken to the laboratory for immediate measurements, and 3–5 analytical replicates were used per plant (p-NPP) bioassay (Olde Venterink 2011). To assess mycorrhizal infection, roots were first cleared and then stained for analysis. Within 12 hours after harvest, fine roots were cut and placed in glass tubes with chloridric acid (HCl) 1 M and kept in 90 ºC water bath for one hour. After this first hour, the HCl solution was renewed and roots kept in water bath for two more hours. The cleared samples were rinsed with tap water, rinsed with 5% HCl and rinsed with water again.

The roots were then stained for 20 minutes in a solution 5% ink (Parker Qink Black, Newell Rubbermaid, Saint Herblain, France) in 5% acetic acid solution, also at 90 ºC following the protocol by Vierheilig et al. (1998). To quantify mycorrhizal colonization rate, root dissections were analysed under magnification and the presence of arbuscles, vesicles or hyphae was recorded. Plant height and stem diameter were measured with a flexible ruler. Water content, aerial dry biomass, root dry biomass and total dry biomass were measured after drying the material at 60°C for three days.

The chemical attributes of the soil used for the experiment are: 11 $mg.dm^{-3}$ resin-P; 19 $g.dm^{-3}$ organic matter; water-pH 5.0; K, Ca, Mg, $H^{+}Al$ = 1.4; 11.0; 9.0 and 22.0 $mmol_c.dm^{-3}$, respectively; Cu, Fe, Mn, Zn = 1.6; 16.0; 20.0 and 0.7 $mg.dm^{-3}$, respectively; 0.17 $mg.dm^{-3}$ B, CEC = 43.4 0 $mmol_c.dm^{-3}$, 49% bases saturation and granulometry of 420, 50 and 530 $g.kg^{-1}$ of sand, silt and clay respectively. Extractable P was measured colorimetrically after extraction with ion exchange resin and then washed with 0.8 M NH_4Cl and 0.2 M HCl. N concentration was measured using the micro-Kjeldahl procedure. Extractable sulfur (S) was measured colorimetrically after extraction with activated charcoal and 0.01M $Ca(H_2PO_4)$. Soil pH was measured in a soil–water suspension (10 g dry soil in 50 ml deionized water) using a Metrohm Herisau pH meter with a Mettler Toledo electrode. Soil organic matter content was determined colorimetrically after extraction for 10 min with 0.667 M sodium dichromate and 5 M sulfuric acid. Soil extractable B was measured after extraction of 10 cm^{-3} dry soil with 20 ml barium chloride 6 mM solution by heating in a microwave at 490 W for 5 min. The B concentration was measured colorimetrically using the azomethine-H method and adsorption at 420 nm on a spectrophotometer (Varian 50 Probe). Extractable Ca, Cu, Fe, Mg, Mn, K, and Zn concentrations were measured by means of atomic adsorption. Extractable Al was measured after extraction with 1 M KCl and titration with NaOH using the phenolphthalein method. All soil chemical characteristics were determined through standard methods at the UNESP Soil Laboratory according to Raij et al. (2001) and Lannes et al. (2020b). At the end of the experiment, one soil sample per pot was collected for determinations of nutrients following the same methods.

2.2. Field Study 1

Field Study 1 was conducted from November 2018 to January 2019 in Selvíria, in the State of Mato Grosso do Sul (20°20’50.65”S 51°24’06.32”O, 344 m elevation), located 11 km on the Northwestern of Glasshouse study area. Soils are classified as dystrophic Red Latosol – Oxisol (Santos et al. 2018). Climate is characterized as Aw (Köppen 1918) – tropical wet with a rainy season generally occurring from November to March and a pronounced dry season from April to October.

From seeds, we cultivated four genotypes under two fertilization treatments and three repetitions. The genotypes were constituted by three lineages (L4, L8 and L12, Appendix 1) – which were selected because they had significantly lower PME activity in the control than in P-fertilized pots in the Glasshouse Study, and a flint maize population (Pop), selected for low technology, genetically variable and equilibrated. The treatments used were control – only water added, or NPK fertilization – 250 kg ha^{-1} of 8(N): 28(P_2O_5): 16(K_2O), yielding 70 kg ha^{-1} P_2O_5. The plants were grown on lines with 3 meters length

each with inter-row distance of 0.90 m. To prevent water stress, all plots were irrigated two to three times a day according to the normal on-farm practice in this area. In the V10 growth stage we measured plant height, plant water content and PME activity using the abovementioned methods.

At the end of the experiment, three top-20 cm soil samples were collected with a 5 cm diameter auger and combined in a composite sample. Samples were air dried, sieved and sent to the UNESP Soil Laboratory for determination of chemical attributes as previously described.

2.3. Field Study 2

A second field study was conducted from April to September 2019 in an area located close to where Field Study 1 took place. We used 13 commercial hybrids (Appendix 2) in three randomized blocks and two treatments: Control – NK addition (250 kg ha^{-1} of 8(N): 0(P_2O_5): 16(K_2O), or NPK fertilization (250 $kg.ha^{-1}$ of 8(N): 28(P_2O_5): 16(K_2O), yielding 70 $kg.ha^{-1}$ P_2O_5). The plants were grown on 6 lines with 5 meters length each with inter-row distance of 0.45 m, in a total density equivalent to 60.000 plants per hectare. To prevent water stress, all plots were irrigated two to three times a day according to the normal on-farm practice in this area. In the V10 growth stage we measured PME activity, number of leaves and plant water content, and at the beginning of the reproductive stage, plant height and high spike insertion height were measured using the abovementioned methods. After harvest, we measured the weight of 100 randomly selected grains.

At the end of the experiment, three top-20 cm soil samples were collected with a 5 cm diameter auger and combined in a composite sample. Samples were air dried, sieved and sent to the UNESP Soil Laboratory under the current methods mentioned in the previous sections.

2.4. Data analyses

The effect of fertilization upon measured variables was analysed through Student t tests and the differences amongst genotypes were assessed through ANOVA followed by Tukey test using IBM SPSS Statistics 20. We applied structural equation modelling (SEM) to investigate the integrated effects of fertilization and genotype identity on PME activity and mycorrhizal colonization and of these on growth variables from the Glasshouse Experiment using Stata 16 (64-Bit). Linear regression analyses were employed to assess the effects of phosphatase activity on growth and productivity parameters. Data were log transformed when necessary to reach normal distribution and homoscedasticity.

3. Results

3.1. Glasshouse Study – Pure lineages in mesocosms

Fertilized pots had significantly higher available phosphorus concentration than unfertilized pots (respectively 71.3±32.5 and 11.7±0.58 $mg.dm^{-3}$, t=10.10, P=0.034), confirming that the fertilization treatment was effective. Phosphorus fertilization did not affect other soil variables, with exception to iron,

whose concentrations also increased in the soil under P fertilization (Fertilized: 34.0±5.6 and Control: 21.3±2.3 $mg.dm^{-3}$, t=13.2, P=0.022).

Root PME activity significantly decreased under P fertilization when all lineages were analysed together (Table 1). Oppositely, P fertilization had a positive influence of stem diameter, aerial dry biomass and root water content. Plant height, number of leaves, total and root dry biomass were not affected by P addition (Table 1).

Six studied lineages (L4, L8, L9, L10, L12 e L14) had higher PME activity in the control than in the fertilized pots, whereas the other eleven lineages (L1, L2, L3, L5, L6, L7, L11, L13, L15, L16 e L17) were not affected by P fertilization (Table 2). Although PME activity generally decreased under P fertilization (Table 1; Table 2), the lineages showed highest differences amongst themselves under P fertilization, as observed in L3 (314±124 µmol pNPP $g\text{-}root^{-1}.h^{-1}$) and L4 (303±34 µmol pNPP $g\text{-}root^{-1}.h^{-1}$), which were significantly lower than L1 (779 ±462 µmol pNPP $g\text{-}root^{-1}.h^{-1}$), L9 (774 ±9 µmol pNPP $g\text{-}root^{-1}.h^{-1}$) and L11 (772 ±305 µmol pNPP $g\text{-}root^{-1}.h^{-1}$) (Table 2).

In general, the lineages did not respond to P fertilization in terms of mycorrhizal colonization rates, with exception to L4, which had higher values under P fertilization (Appendix 3).

Structural equation modelling revealed that P fertilization negatively influenced root PME activity and that the type of lineage affected both mycorrhizal colonization rates and root PME activity. Root PME activity negatively affected number of leaves, stem diameter, root, aerial and total plant biomass, whereas mycorrhizal colonization positively affected plant height, number of leaves, aerial and total plant biomass (Figure 2).

3.2. Field Study 1 – Pure lineages in the field

The overall average root PME activity of the lineages cultivated in the field was higher in the controls that under NPK fertilization, but no lineage individually has shown such resoponse (Table 2). Root PME activity was generally higher in the field than in the glasshouse study (Table 2; Figure 3).

3.3. Field Study 2 – Commercial hybrids in the field

There was generally no effect of fertilization on root PME activity for the studied hybrids, with exception to hybrid 11, which had higher PME activity in the fertilized than in control plants (Table 3). No differences in PME activity were observed amongst the hybrids (Table 3).

The hybrids 1 and 9 had higher weight of 100 grains in the fertilized than in the control plants. For plant height, the hybrids 1, 2, 3, 8, 9, 12 and the general mean were higher in the control than in fertilized plants. The high spike insertion height of hybrid 2 was higher in the control, and the opposite was observed for

the hybrids 4 and 10 ($P<0.05$). The weight of 100 grains and the main spike height were different amongst the hybrids in both treatments, and plant height only differed in the control plots (Table 3).

Root PME activity positively influenced the weight of 100 grains of the hybrids in the control plots (Figure 4), but it did not influence other variables (Appendix 4).

3.4. Soil characteristics

Soil P concentrations were not significantly different amongst the three experiments (Table 4). Nitrogen, copper and manganese concentrations were different in all experiments. Organic matter and potassium concentrations were higher in the field studies than in the glasshouse study, and the opposite was observed for pH. Calcium and magnesium concentrations were higher in the Field Study 2 than in the other experiments, and zinc concentration was lower in the Glasshouse Study that in the Field Study 2 (Table 4).

4. Discussion

The observed higher biomass and stem diameter under P fertilization in the glasshouse study were expected results (Table 1), since higher P availability yields a general better plant development, due to the essentiality of this nutrient for maize metabolism (Plénet et al. 2020).

Higher P availability inhibits root PME activity (Olde Venterink and Güsewell 2010) because the plant does not need to produce the enzyme when its product is abundant, which explains the lowest activities in P-fertilized plants in both studies using pure lineages (Table 2). For maize (*Zea mays*), however, the effect of P addition upon metabolism varies according to the genotype and to its susceptibility to soil P deficiency (Gaume et al. 2001). The genetic regulation system normally acts to avoid unnecessary expression of genes in specific organs and in specific time periods. Any deviation from this pattern indicates variation in the regulatory genes system, as observed in hybrid 11 (Table 3), which had higher root PME activity under P fertilization, similarly to the genotype studied by Wei et al. (2020) that detected higher root PME activity under NPK + maize straw treatment in comparison to unfertilized control plants. Most lineages investigated in this study did not respond significantly to P addition, showing that for these experimental genotypes, genes acting on the regulation of root PME activity are generally not sensitive to soil P availability.

The lineages 4, 8 and 12 showed different responses to P fertilization when cultivated in the glasshouse and in the field. While their PME activity values were higher in the control than in the fertilized pots in the glasshouse, such differences were not detected in the field (Table 2). Higher activities were always detected in the field than in the glasshouse in both treatments (Table 2; Fig. 3). The higher soil nitrogen availability in the field when compared to the glasshouse (Table 4) may have stimulated the activity of the enzyme since it is N-rich and therefore highly controlled by N (Olde Venterink and Güsewell 2010). Another possible explanation for the higher PME activity observed in the field resides in the presence of

neighbouring plants in the field, whose root contact stimulates root PME activity, as shown by Lannes et al. (2020a) for Cerrado wild plants.

When grown alone in the glasshouse pots, the lineages presented reduced PME activity in relation to the field presumably because it tends to invest more nitrogen in plant development and therefore have less N to employ in other strategies, as PME activity. In the glasshouse control pots, however, the lineages invested more in PME activity due to the lower soil P availabilities, at the cost of lower growth (Table 1), which suggests a tradeoff between high PME activity and nitrogen economy. The overall negative influence of PME activity on plant growth characteristics of lineages growing in the glasshouse could also be observed by the negative effects of PME activity on growth variables, as demonstrated by the structural equation modelling (Fig. 2). These observations point to a low availability of N in the soil as a limiting factor for plant growth when it invests in PME activity and does not have sufficient N for growth. On another hand, mycorrhizal colonization effects upon growth were positive, showing the importance of this symbiosis for plant development, independently on the soil N and P statuses.

Lu et al. (2016) promoted the superexpression of the genes OsPAP10a and OsPAP10c in genetically modified rice and Wang et al. (2009) inserted the *Arabidopsis thaliana* gene AtPAP15 in genetically modified soybeans aiming at increasing root PME activity. Although they observed a better efficiency on organic-P use in soils with low inorganic P availability, the yield was still lower in comparison to the unmodified genotypes. These experiments did not consider nitrogen as an important regulation factor for PME activity. We reinforce that high availability or use efficiency of N is necessary to convert high PME activity in productivity gain, given that this enzyme is highly N demanding. Nitrogen availability and PME activity acting to release P seem to colimit grain filling and plant development because as root PME activity increases, more internal nitrogen in used, reducing the availability of N for plant metabolism and thus affecting plant growth. Therefore, we suggest that for optimum productivity and/or growth increase in maize, it is necessary that N availability and PME activity increase concomitantly, independently of the soil type. The application of this practice could support a more sustainable maize production system, which could be even more effective if genotypes with N-fixing capacity are used, as in association with diazotrophic bacteria, mainly *Azospirillum brasilense* that can provide from 29–82% of the overall N absorbed by maize (van Deynze et al. 2018).

The differences in PME activity found amongst the lineages show that genetic variability for this character exists (Table 2), agreeing with results shown by Machado and Furlani (2004), which evaluated six genotypes (three common and three improved varieties) and identified one genotype with significantly a lower PME activity in comparison to the others. The occurrence of genetic variability was also detected by Chen et al. (2008), who estimated the broad-sense heritability as 52.7% for PME activity and as 90.9% for P absorption efficiency in maize. Chen et al. (2008) also reported that the two tested parentals differed significantly for PME activity, with the P-deficiency tolerant genotype having higher values than the P-deficiency susceptible genotype.

No differences were detected in root PME activity of the commercial hybrids investigated, but the comparison of their PME activity under fertilization to the field fertilized lineages (1376 ± 478 and 930 ± 330 μmol pNPP g-root^{-1}.h^{-1} respectively, F = 13.06, P = 0.001) shows that the indirect selection resulting from genetic improvement tends to select low PME activity genotypes, which is corroborated by the high phenotypic and genetic potential of the lineages. This makes possible the generation of simple hybrids with high variability for this character, considering that PME activity is a quantitative character with 33 genes involved on its control (González-Munoz et al. 2015).

The positive effect that PME activity exerts on the weight of 100 grains in the P unfertilized hybrids (Fig. 4) demonstrates, for the first time, the importance of the activity of this enzyme for productivity enhancement in maize under low P conditions and reinforces the need to consider this variable for P acquisition in natural, non-P fertilized agricultural systems.

Since it not common that genetic improvement companies test their hybrids under natural unfertilized conditions and due to the high number of simple hybrids currently available in the market, it is possible that some of these genotypes already have high N-use efficiency and high PME activity that could make them suitable for more sustainable systems without problems associated to productivity loss. As an example, Wei et al. (2020) demonstrated that there were no significant productivity losses after a 20% P fertilization reduction, outlining the compensation of the inorganic P addition by maize straw and root PME increase.

Based on previous studies performed with purple acid phosphatases (PAPs) (Tran et al. 2010; Gonzalez-Munoz et al. 2015), we suggest that lineages that diverged in PME activity in response to soil P addition (as lineages 1, 9 and 16) are feasible genotypes for functional analyses of the genes PAPs. Following the recommendations of González-Munoz et al. (2015), this can facilitate the use of PAPs as targets for direct selection and manipulation by genetic improvement efforts. Other lineages that diverged in PME activity values for the different soil P availability conditions can be used for breeding aiming at verifying the occurrence of heterosis for root PME activity, which would allow a better decision making about the viability of a genetic improvement program targeting the activity of this enzyme, both for use under traditional or more sustainable organic agriculture.

With this work, we show that there is possibility of success on the selection of genotypes of maize that are naturally adapted to low soil phosphorus availability, and concomitantly of genotypes that have high root PME activity under phosphorus fertilization as a way to reduce the amount of inorganic phosphorus needed to grow maize in the Cerrado. These will help farmers that do not base their maize production on high technology, and will also benefit conventional farmers by reducing the amount of P needed for production. Future researches should specifically investigate the relationship between nitrogen availability and root PME activity in maize, by targeting on tests of simple hybrids without P addition. Considering that the current increases in carbon and nitrogen due to human activities are not followed by increases in phosphorus availability (Penuelas et al. 2020) and that such unbalanced stoichiometries can cause losses for agriculture, we recommend the generation of genetic variability for root PME activity

together with improving the nitrogen absorption and use efficiency in maize aiming at productivity increase in more sustainable production systems.

5. Conclusions

Root phosphatase activity and mycorrhizal colonization rates in maize cultivated in Brazil are dependent on the genotype and important for plant development. Due to its high genetic potential, and for positively influencing grain productivity in low P soils, the insertion of root phosphatase activity in programs of maize genetic improvement aiming at a higher P efficiency is promising for the development of genotypes adapted to more sustainable production systems in phosphorus impoverished areas.

Declarations

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Author’s contributions

LLS, JACA and LSL developed and framed the research question. LLS and JACA conducted the experiments. LLS and LSL analysed the data. LLS and LSL wrote the paper. JACA and KLM contributed to paper writing. LSL coordinated the overall project. All authors read and approved the final manuscript.

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Conflicts of interest/Competing interests

The authors declare no competing interests

Availability of data and material

The datasets generated and/or analysed during the current study are available at the Unesp Institutional Data Repository

Code availability

Not applicable

Ethics approval

Not applicable

Consent to participate

All authors consent to participate in this manuscript

Consent for publication

All authors consent to publication of this manuscript

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Tables

Table 1

Mean values, standard deviations (in parentheses), and P values associated to t tests checking the effect of P fertilization (200 mg P kg^{-1} soil) on measured variables in 17 lineages in the V9 growth stage of maize (*Zea mays*) from the Glasshouse Study. N=3.

Variables	Control	Fertilized	P
PME activity (µmol pNPP g-$root^{-1}$ h^{-1})	610 (224)	505 (207)	0.003
Height (cm)	64 (11)	61 (16)	0.170
Number of leaves (unit)	9.5 (1.2)	10.2 (1.8)	0.055
Stem diameter (cm)	8.5 (2.2)	9.8 (1.8)	<0.001
Root biomass (g)	1.46 (0.78)	1.74 (1.28)	0.844
Aerial biomass (g)	2.56 (1.04)	3.24 (1.65)	0.039
Total biomass (g)	4.03 (1.60)	4.99 (2.71)	0.169
Water content (%)	88.31 (2.46)	89.50 (1.52)	0.004

Table 2

Mean values, standard deviations (in parentheses) and significance values of the P fertilization effect (200 mg P kg^{-1} soil) on PME activity (µmol pNPP g-$root^{-1}$ h^{-1}) in 17 pure lineages and in the Flint maize population (*Zea mays*) in the Glasshouse Study and in the Field Study 1. The effects of P fertilization were tested by means of Student t tests and the differences amongst the lineages were tested through ANOVA followed by Tukey tests. Different letters indicate significant differences amongst the lineages within treatments. (P<0.05), N=3.

Lineages	Glasshouse Study					Field Study 1		
	Control		P Fertilization		P	Control	NPK Fertilization	P
L1	552 (163)	a	779 (462)	bc	0.518	.	.	.
L2	526 (108)	a	391 (120)	abc	0.200	.	.	.
L3	448 (112)	a	314 (124)	a	0.248	.	.	.
L4	494 (109)	a	303 (34)	a	0.030	1754 (368)	1386 (359)	0.148
L5	504 (87)	a	452 (129)	abc	0.555	.	.	.
L6	531 (132)	a	649 (233)	abc	0.494	.	.	.
L7	491 (51)	a	421 (153)	abc	0.404	.	.	.
L8	691 (114)	a	392 (39)	abc	0.007	1173 (260)	1385 (916)	0.720
L9	933 (45)	a	774 (9)	c	0.003	.	.	.
L10	712 (12)	a	506 (52)	abc	0.005	.	.	.
L11	861 (747)	a	772 (305)	bc	0.873	.	.	.
L12	712 (129)	a	436 (96)	abc	0.044	2649 (1351)	1351 (208)	0.175
L13	469 (26)	a	432 (95)	abc	0.510	.	.	.
L14	617 (109)	a	356 (40)	ab	0.010	.	.	.
L15	637 (220)	a	527 (17)	abc	0.461	.	.	.
L16	601 (139)	a	607 (138)	abc	0.973	.	.	.
L17	594 (233)	a	478 (67)	abc	0.595	.	.	.
Flint population	.	.	.		.	1994 (536)	1637 (652)	0.198

Total	610 (224)	.	505 (207)	0.003	1913 (720)	1500 (569)	0.046
P and F (ANOVA)	P=0.079		P<0.001		P=0.069	P=0.801	
	F=1.790		F=4.051		F=2.831	F=0.333	

Table 3

Mean values and standard deviations of root PME activity (µmol pNPP g-root^{-1} h^{-1}), weight of 100 grains (g), plant height (cm) and high spike insertion height (cm) of 13 simple commercial hybrids of maize cultivated in the Field Study 2 under two treatments: Control – NK addition (250 kg ha^{-1} of 8(N): 0(P_2O_5): 16(K_2O), or NPK fertilization (250 kg ha^{-1} of 8(N): 28(P_2O_5): 16(K_2O), yielding 70 kg ha^{-1} P_2O_5). The effects of fertilization upon measured variables were tested by means of Student t tests (N=3) and indicated by asterisks when significant (* P<0.05, ** P<0.01. Differences among hybrids were analysed through ANOVA followed by Tukey test (N=3). Different uppercase letters indicate differences in the control treatment and different lowercase letters show differences under P fertilization. (P<0.05). The same rule applies for upper- and lowercase P and F values.

Hybrid	Treatment	PME activity	Weight of 100 grains	Plant height	Spike insertion height
H1	Control	1012 (341)	35.67 (2.8) bc	222 (5.3)** bcde	127 (12.2) cd
H1	P-fertilized	885 (138)	40.85 (1.1)* C	200 (2.0)	121 (5.0) B
H2	Control	1168 (430)	35.40 (0.6) bc	245 (4.7)** e	130 (11.2)* d
H2	P-fertilized	1356 (548)	36.26 (2.0) ABC	212 (23.5)	114 (20.0) AB
H3	Control	835 (194)	37.93 (5.9) c	215 (2.0)* abcd	96 (5.8) a
H3	P-fertilized	641 (77)	40.73 (1.4) C	202 (8.7)	103 (3.8) AB
H4	Control	840 (435)	31.37 (3.4) abc	195 (14.5) a	95 (9.8) a
H4	P-fertilized	661 (217)	30.88 (2.2) AB	197 (15.1)	106 (15.5)* AB
H5	Control	1067 (244)	35.22 (7.2) bc	227 (9.8) bcde	119 (12.7) bcd
H5	P-fertilized	929 (374)	37.36 (1.6) BC	217 (14.5)	119 (4.5) B
H6	Control	799 (310)	31.93 (0.3) abc	214 (18.8) abc	112 (25.2) abcd
H6	P-fertilized	885 (60)	30.50 (6.6) AB	205 (6.0)	118 (10.8) B
H7	Control	1011 (371)	34.08 (2.8) abc	206 (16.5) ab	96 (14.4) a
H7	P-fertilized	842 (69)	38.01 (4.0) BC	202 (9.4)	99 (3.5) A
H8	Control	774 (290)	26.93 (1.8) ab	214 (6.4)** abc	124 (1.0) cd
H8	P-fertilized	1199 (429)	33.26 (4.4) ABC	196 (8.7)	120 (13.6) B
H9	Control	782 (620)	28.65 (1.3) abc	231 (7.5)** bcde	106 (20.5) abc
H9	P-fertilized	1090 (542)	32.25 (1.9)* ABC	200 (7.0)	121 (8.3) B
H10	Control	575 (85)	30.37 (2.1) abc	210 (15.0) abc	96 (10.4) a
H10	P-fertilized	715 (213)	29.03 (0.4) AB	201 (8.5)	105 (8.5)*AB
H11	Control	727 (90)	24.88 (1.8) a	210 (19.9) abc	99 (13.8) ab

H11	P-fertilized	947 (66)*	27.28 (3.3) A	193 (27.2)	104 (7.0) AB
H12	Control	627 (284)	30.65 (1.1) abc	240 (10.0)** de	132 (4.2) d
H12	P-fertilized	1150 (368)	29.82 (1.4) AB	215 (24.0)	126 (13.6) B
H13	Control	956 (190)	29.49 (2.2) abc	232 (16.7) cde	121 (9.5) bcd
H13	P-fertilized	1150 (87)	31.77 (4.8) ABC	216 (14.5)	118 (13.2) B
Total	Control	860 (313)	31.74 (4.5)	220 (24.7)**	112 (22.1)
Total	P-fertilized	930 (330)	33.69 (5.1)	204 (25.9)	113 (16.8)
P and			p=0.001/P<0.001	P<0.001	P<0.001/P<0.001
F (ANOVA)			f=4.19/F=5.87	f = 6.90	f=9.87/F=4.57

Table 4

Mean values, standard deviations (in parentheses) and P values for soil characteristics in the three experiments performed. Differences amongst the experiments were tested by means of ANOVA followed by Tukey test (N=3). Different letters indicate significant differences in the concentration of the given parameter (P<0.05).

	Glasshouse Study	Field Study 1	Field Study 2	F	P
N ($g.kg^{-1}$)	0.93 (0.07) a	1.39 (0.09) b	1.70 (0.08) c	117.55	<0.001
P - resin ($mg.dm^{-3}$)	41.5 (38.6)	40.0 (13.5)	36.3 (7.3)	0.03	0.970
MO ($g.dm^{-3}$)	19.3 (1.03) a	24.7 (1.15) b	26.3 (2.08) b	31.93	<0.001
pH ($CaCl_2$)	5.03 (0.1) b	4.63 (0.15) a	4.73 (0.12) a	13.59	<0.001
K ($mmol_c.dm^{-3}$)	0.78 (0.08) a	5.06 (0.67) b	4.13 (0.71) b	105.39	<0.001
Ca ($mmol_c.dm^{-3}$)	12.3 (0.82) a	10.0 (1.73) a	16.3 (1.15) b	23.34	<0.001
Mg ($mmol_c.dm^{-3}$)	10.0 (0.63) a	9.7 (0.58) a	12.7 (1.53) b	10.79	<0.001
Al ($mmol_c.dm^{-3}$)	1.67 (0.82)	3.33 (1.53)	2.33 (0.58)	2.90	0.110
B ($mg.dm^{-3}$)	0.15 (0.05)	0.19 (0.03)	0.17 (0.04)	0.80	0.480
Cu ($mg.dm^{-3}$)	20.8 (1.3) a	52.7 (1.5) b	62.7 (2.5) c	725.75	<0.001
Fe ($mg.dm^{-3}$)	27.7 (7.9)	22.3 (1.1)	26.0 (0)	0.81	0.470
Mn ($mg.dm^{-3}$)	24.4 (1.5) a	29.8 (0.5) b	41.6 (0.4) c	221.11	<0.001
Zn ($mg.dm^{-3}$)	0.72 (0.04) a	3.77 (1.69) ab	6.77 (5.58) b	5.01	0.030

Figures

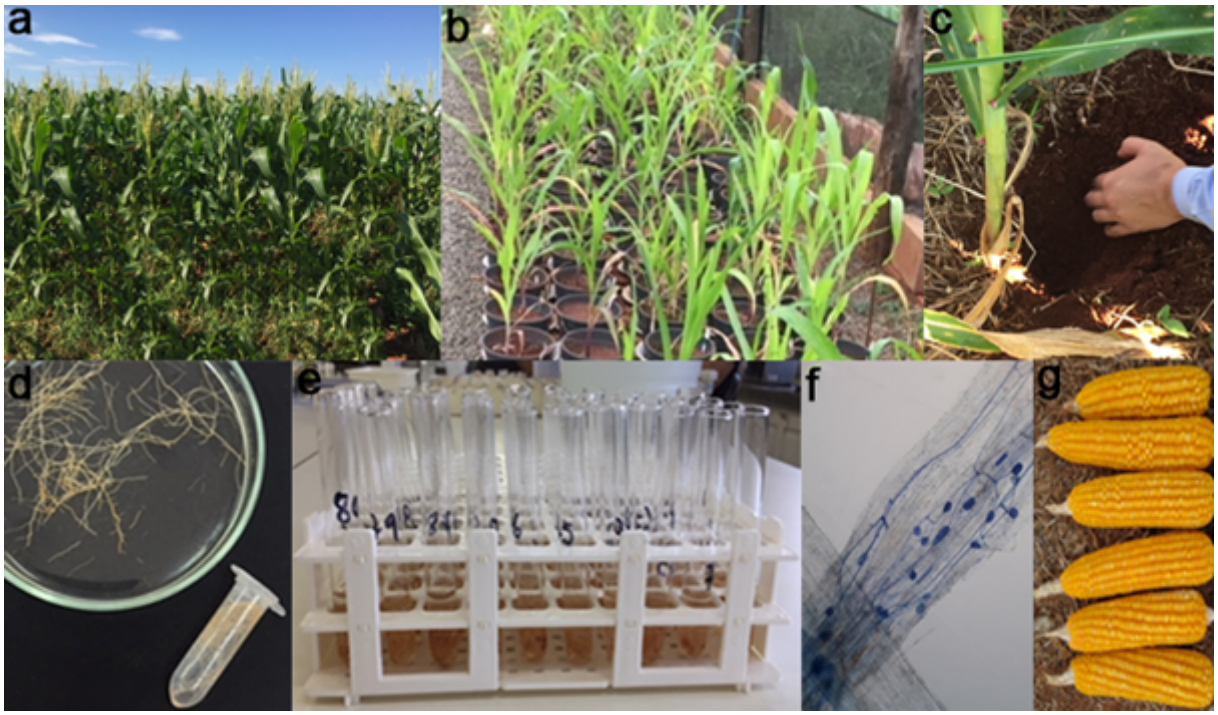

Figure 1

Maize plants growing in the field (a,c) and in the glasshouse (b) for assessment of the effects of genotype on phosphatase activity (d,e) and mycorrhizal colonization rates (d,f) and of these upon productivity (g) in Brazil. Photo credits: a, b, c – Luciola S Lannes; d, e, f – Lucas Lopes e Silva; g – João Antonio da Costa Andrade.

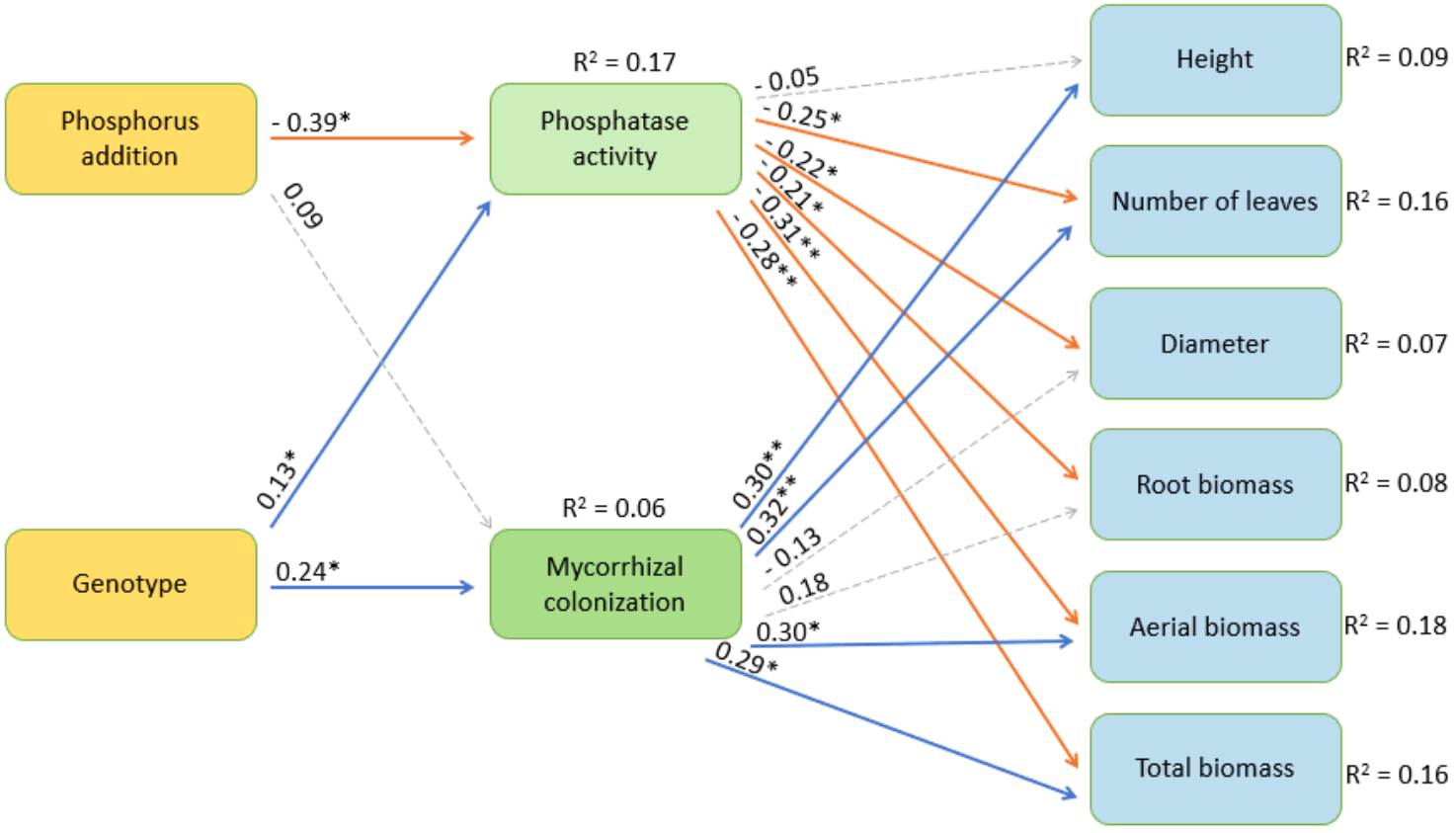

Figure 2

Structural equation model (SEM) showing the effects of P fertilization and lineage identity upon root PME activity and mycorrhizal colonization and of these upon plant height, number of leaves, stem diameter, root, aerial and total plant biomass in 17 pure lineages of maize (Zea mays) cultivated in the Glasshouse Study. Numbers next to the arrows indicate the standardized values (scaled by the standard deviations of the variables). Blue and red arrows respectively indicate significant positive and negative relationships. Grey dashed arrows show non significant interactions (P>0.05). *, P<0.05; **, P<0.01, *** P<0.001. Goodness of fit of the SEM: p $\chi 2$ = 0.152 (a good model fit indicating that the fit is clearly not significantly different from the theoretical model).

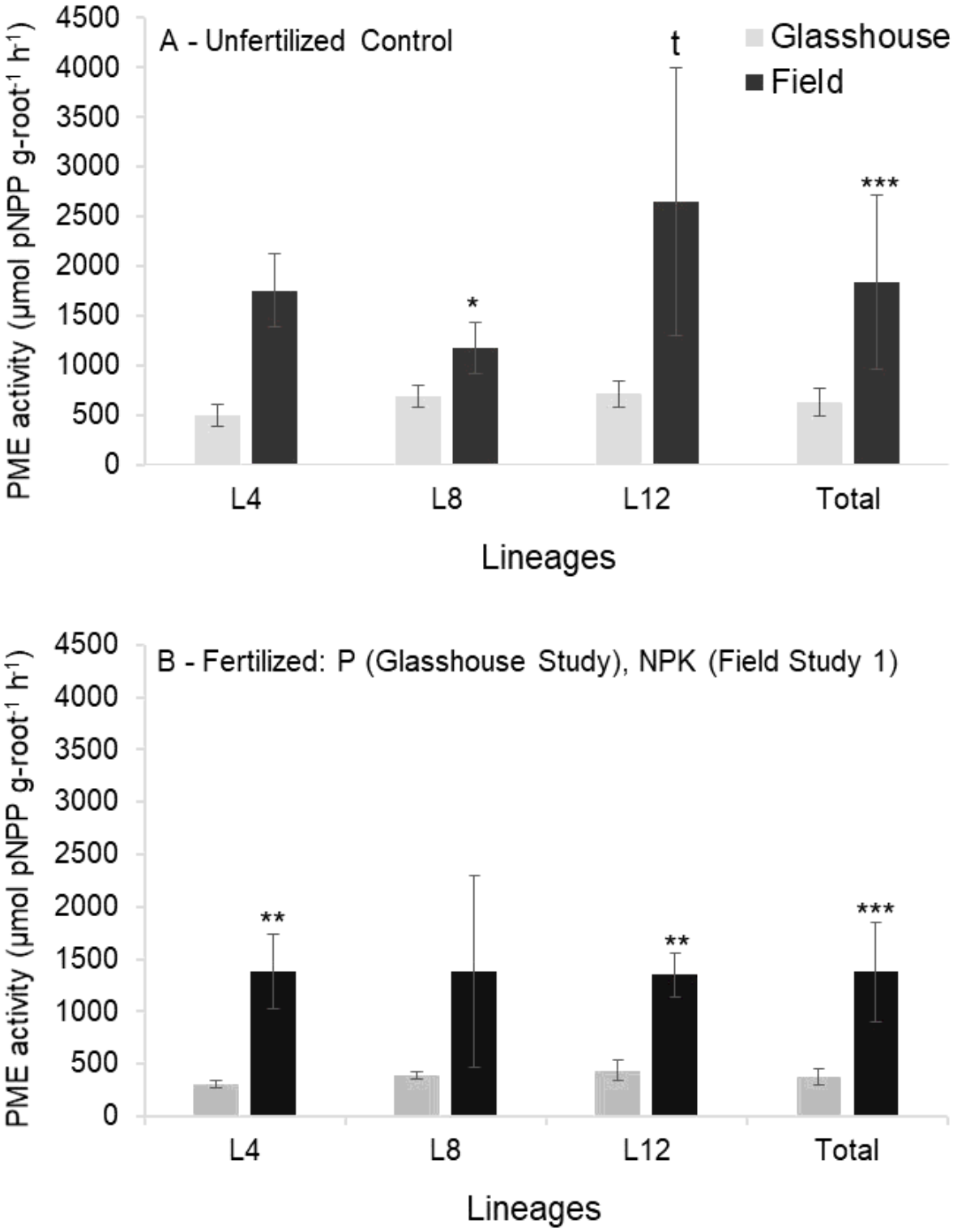

Figure 3

Root PME activity of four pure individual lineages (L4, L8 and L12) and analysed together (Total) in the Glasshouse Study and in the Field Study 1 in the unfertilized controls and in the fertilized treatments. Means, standard deviations and levels of significance resulting from Student t tests are shown. t, 0.10>P>0.05; *, P<0.05; **, P<0.01, *** P<0.001.

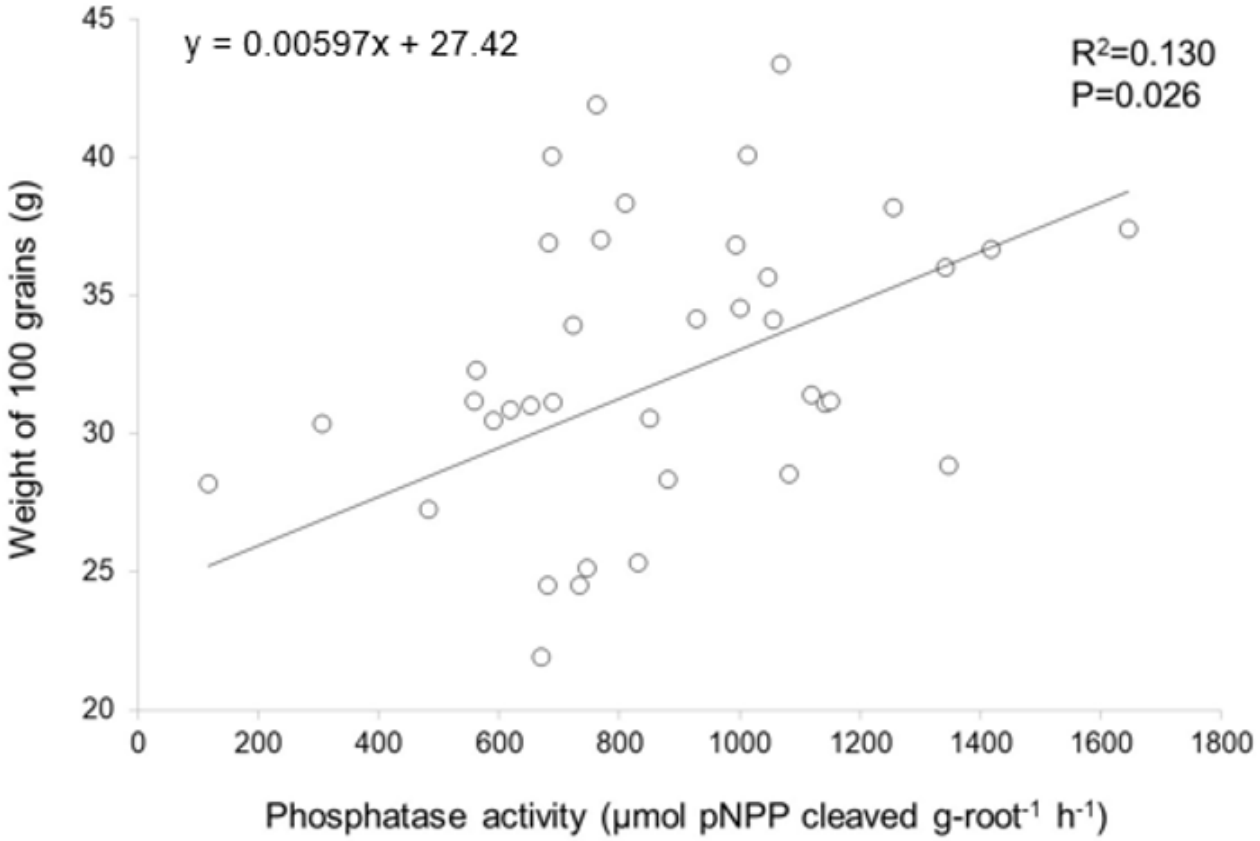

Figure 4

Effect of root PME activity on weight of 100 grains in P-unfertilized control plots in 13 commercial hybrids of maize (Zea mays) cultivated in the field. N= 39.

Supplementary Files

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- Appendix.docx