

Decline of seedling phosphorus use efficiency in the heterotic pool of flint maize breeding lines since the onset of hybrid breeding

Xuelian Li¹  | Melissa Mang¹ | Hans-Peter Piepho² | Albrecht Melchinger³ | Uwe Ludewig¹ 

¹Institute of Crop Science, Nutritional Crop Physiology, University of Hohenheim, Stuttgart, Germany

²Institute of Crop Science, Biostatistics, University of Hohenheim, Stuttgart, Germany

³Institute of Plant Breeding, Seed Science and Population Genetics, Plant Breeding, University of Hohenheim, Stuttgart, Germany

Correspondence

Uwe Ludewig, Institute of Crop Science, Nutritional Crop Physiology, University of Hohenheim, Fruwirthstr. 20, 70593 Stuttgart, Germany.
Email: u.ludewig@uni-hohenheim.de

Funding information

The German Research Foundation, Grant/Award Number: 328017493/GRK 2366; the China Scholarship Council, Grant/Award Number: 201606350143; UNIVERSITAET HOHENHEIM

Abstract

Improved management and breeding increased maize (*Zea mays* L.) yields over the last century, but nutritional efficiency was usually not the focus. This study investigates whether old and recently released flint and dent maize seedlings vary in the phosphorus (P) acquisition and utilization. P use efficiency (PUE) and related traits were measured and compared at two P levels in a calcareous soil. PUE and P acquisition efficiency (PAE) from founder flints to elite flints declined over the last decades. This was associated with smaller root systems, reduced ability to exploit external P, decreased rhizosphere pH and shorter root hairs in low P. Comparing flints with doubled haploid landraces (DH_LR), old and elite dents and hybrids revealed that dents started to acquire exogenous P earlier and had improved PUE. Most DH_LRs had similar PUE as elite flints. When evaluating root traits associated with P efficiency, seed P was also critical, and it is important to stack different root traits to optimize PUE, P utilization efficiency (PUtE) and PAE in breeding programmes. The root hair length, the ability to acidify the rhizosphere and the root diameter in flint and dent pools may be utilized to improve P use in maize germplasm.

KEYWORDS

exudates, nutrient stress, phosphate, rhizosphere, rhizotron, root architecture

1 | INTRODUCTION

Of all essential macro-elements for plants, phosphorus (P) is typically least bioavailable in soils, despite high total P amounts within soils. Almost all P in soils is found in fully oxidized form as orthophosphate (P_i), but due to slow soil processes such as adsorption, precipitation and immobilization (often in organic molecules), the free P_i concentration even in agricultural soil solution

is rarely above 10 μM and sparingly soluble P-forms are converted into insoluble forms with time (Bielecki, 1973). This low P_i concentration is more typical for micro-nutrients that are required by orders of magnitude less. If available, P is readily taken up by the roots as (di-)hydrogen phosphate (H_2PO_4^- or HPO_4^{2-}) and the P_i concentrations within plant cells are approximately 5 to 20 mM (Raghothama & Karthikeyan, 2005). Although many soils have large reserves of total P, the inorganic P (P_i) and organic P (P_o)

Xuelian Li and Melissa Mang these authors contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2021 The Authors. *Journal of Agronomy and Crop Science* published by Wiley-VCH GmbH

pools of most soils are sparingly soluble and little mobile, especially at acidic and slightly basic pH (Akhtar et al., 2008).

Ensuring stable food and feed production even with dwindling productive agricultural zones with P as a finite, but an essential resource, requires resource-saving production and nutrient efficient crops that cope with these soil properties (Wang et al., 2010; Wissuwa et al., 2009). Several definitions and calculation methods are common for crop P use efficiency (PUE), an indicator of the ability of plants to produce yield or biomass under particular P conditions (Batten, 1992; Lynch, 1998; Moll et al., 1982). PUE is often separated into P acquisition efficiency (PAE) and P utilization efficiency (PUtE). PAE is the ability of plants to capture P from soils; while PUtE is the ability to produce biomass or yield using this acquired P. The P efficiency of the plant can be enhanced by improving the acquisition and/or utilization of P (Manske et al., 2001; Parentoni & Souza Júnior, 2008; Shenoy & Kalagudi, 2005).

Plants use diverse strategies to increase P acquisition and/or improve internal P utilization. The below-ground strategies to increase P acquisition can be divided into morphological traits, chemical modifications of the rhizosphere and microbial interactions. Root morphological strategies include vigorous root branching, increased frequency and length of root hairs, and shallow root systems with preferential root development in nutrient-rich topsoil, to access a large soil area (Haling et al., 2018; Postma et al., 2014; Vance et al., 2003). Additionally, plant roots can acquire P by chemical modification of rhizospheres, such as exudation of H^+ , sugars, organic anions, amino acids and secondary metabolites (Hinsinger, 2001; Pang et al., 2018; Richardson & Simpson, 2011). Furthermore, P deficiency can induce the establishment of symbioses with arbuscular mycorrhizal fungi to help access P from the labile soil P pool (Irshad et al., 2012; Smith et al., 2011; van der Heijden et al., 2015).

There is substantial evidence for coordination and trade-offs among root functional traits among species and environments (Lynch, 2011; Ma et al., 2018; Reinhart et al., 2012; Wen et al., 2019). Among the major crops, maize and wheat with fibrous root systems respond to low soil P availability by altering root size and architecture. Meanwhile, legumes tend to modify the chemistry of the rhizosphere (Lyu et al., 2016). Rapeseed, barley and potato have evolved specific traits of root morphology and root exudation that enhance their P uptake capacity under low P conditions (Wang et al., 2015). The root diameter exerts a strong influence on root trait variation across plant species, growth forms and biomes (Lyu et al., 2016; Ma et al., 2018; Wen et al., 2019). Maize seedlings performed differently in different soil substrates (Liu et al., 2017). Erel et al. (2017) compared maize hybrids grown in neutral and alkaline soil and found that the soil type had a strong effect on PAE. In the neutral soil, the best genotypes were characterized by topsoil exploration, but in the alkaline soil, the 'mining strategy' was optimal.

Recently, there is also increasing evidence that root functional traits changed along with the evolution history (Ma et al., 2018; Reinhart et al., 2012). Due to long-term agricultural practices, especially high fertilization and long-term organic residue accumulation, the total P pool per hectare increased steeply between 1900 and

2010 (Sattari et al., 2012; Zhang et al., 2017). As modern varieties have often been selected under high-nutrient input conditions to obtain a high yield, there is reasonable concern that beneficial traits for uptake under limited P supply are getting lost in elite varieties or even already got lost. Genes or traits related to the efficient nutrient acquisition at low P availability may provide a trade-off when all nutrients are amply available, as plant adaptive traits to nutrient deficiency often result in additional carbon costs (Wang et al., 2010; Wissuwa et al., 2009). By comparing older varieties or landraces with modern lines, loss of root traits contributing to P uptake was already reported in wheat cultivars (Zhu et al., 2001). However, modern cultivars that were selected through conventional breeding approaches for better yield may alternatively unintentionally be better adapted to soil P fluctuations and acquire more P from P-rich zones. For example, modern soya beans have shallow root architecture and higher PAE because they can acquire more P from the soil P-rich surface zone. In contrast, wild soya bean genotypes have deep root architectures and lower PAE, and the root architecture and PAE of semi-wild soya bean are intermediate between modern cultivars and wild soya bean (Zhao, 2004). Furthermore, different maize genotypes (bred in P-rich and P-poor environments, respectively) performed differently in homogeneous and heterogeneous P soils and exhibited different root traits, as well P uptake strategies. The genotypes bred in the P-poor environments showed higher phosphatase activity as well higher mycorrhizal colonization within homogenous P soils compared with genotypes bred under P-rich environments (Li et al., 2019).

In maize, efficient P use is critical between planting and the six-leaf stage, when the young root is still too small to sustain shoot nutrient supply. P deficiency during that stage reduced grain yield (Barry & Miller, 1989). Already after the first week of seedling development, most stored P in the form of phytate was completely hydrolysed and was remobilized for P nutrition, while the uptake of exogenous P in maize was delayed and started only at around the fifth day (Nadeem et al., 2011). Maize seedling development was reported to be initially independent of exogenous P supply; within the first three weeks of growth, losses of up to 40% of seed P were reported (Nadeem et al., 2012).

In this study, we initially selected 16 representative maize genotypes from the flint heterotic pool of a public temperate maize breeding programme that spans the breeding progress since the onset of hybrid breeding from the 1950s to 2010s (Hölker et al., 2019). We hypothesized that the increase in biomass in modern elite varieties is associated with increased PUE, PAE and/or PUtE, compared to old genotypes and landraces. We additionally hypothesized that modern elite genotypes lost some beneficial root-related traits, especially under limited P supply, because of selection under ample P supply. Plants were grown in a calcareous soil-sand mixture with two P application levels and were harvested after three weeks. Moreover, rhizoboxes with observation windows enabled the collection of root physiological traits, such as exudates, and the analysis of root morphology crucial for P acquisition and P utilization. Our analysis was then expanded to doubled haploid landraces and dent

lines from different decades for a broad overview of major germplasm pools (flint and dent), as well as three hybrids (derived from crosses of studied flint and dent lines). We finally hypothesized that high PUE under high P supply is associated with trade-offs in root traits that are beneficial for P acquisition under P limitation, with the root length and root hair length exerting a major influence on other root traits across genotypes.

2 | MATERIALS AND METHODS

2.1 | Plant materials

Thirty four maize genotypes were tested in this study, including 16 flint lines, seven doubled haploid landrace (DH_LR) lines from the flint pool, eight dent lines and three hybrids. The 16 flint lines covered a total of 67 years of hybrid breeding at the University of Hohenheim: four flint founders (FL Flint) EP1, 1,105, F2 and F7; early elite flint lines (EL Flint1, 1,102, 1,107, 1,278, 5,113), moderately old EL Flint2 (5,250, 5,267, 5,271, F012) and most recent EL Flint3 lines (F030, F047, F160, F142). The flint founder lines were registered between the late 1940s and 1974 and played a vital role during the beginning of hybrid breeding in Europe (Cartea et al., 1999; Messmer et al., 1992). The elite flint lines cover a period from the 1970s to 2016 and were among the most successful lines of the UHOH maize breeding programme in their respective time period. Seven DH_LR lines were produced from four different landraces, namely lines SM1, SM2, SM3, SF1, SF2, WA1 and CG1 from the Romanian population Satu Mare, the German population Strenzfelder, the French population Wallis and the Swiss population Campan Galade, respectively. The old dent lines B73 and Mo17 (Old Dent) are typical representatives of the BSSS \times Lancaster heterotic pair. The six elite dent inbred lines (EL Dent) were P024, P040, S072, VD03, P330 and P415. Three hybrids of the studied flint and dent lines (Hybrid) P330 \times F047, P330 \times F142, P415 \times F142 were crossed in 2016. The detailed background of these genotypes is given in Supplementary Table S1.

2.2 | Plant growth conditions

A long-term stored nutrient-poor subsoil (pH (CaCl₂) 7.6, C_{org} <0.3%, CaCO₃ 30%) was used, of which information is found in Supplementary Table S2. In both treatments, the soil was fertilized with 200 mg N (NH₄NO₃), 200 mg K (K₂SO₄), 100 mg Mg (MgSO₄·7H₂O), 2 mg Fe (EDTA-Fe-2Na), 2.6 mg Zn (ZnSO₄·0.7 H₂O) and 1 mg Cu (CuSO₄·0.5H₂O) per kg dry soil to obtain a nutrient-rich soil substrate. Then, the soil was mixed with 30% (w/w) quartz sand for improved soil texture and to facilitate root collection. Phosphorus was added to the soil as Ca(H₂PO₄)₂·H₂O, with a rate of 32 mg P kg⁻¹ soil for the low P treatment; the high P treatment received 132 mg P·kg⁻¹. The P availabilities in these mixtures were determined by CAL extraction (9.8 mg·kg⁻¹ for LP and 61.8 mg kg⁻¹

for HP, respectively) and bicarbonate extraction (Olsen-P, 6.6 mg·kg⁻¹ for LP and 60.2 mg·kg⁻¹ for HP, respectively). After adding the nutrient solution, the soil-sand substrate was sieved through 5 mm before potting. Soil moisture was set to 70% of the soil water holding capacity and gravimetrically adjusted every 2 days.

The experiment was conducted in a growth chamber at the University of Hohenheim, Stuttgart, Germany (48°42'44"N, 9°12'30"E). Seeds were surface-sterilized by rinsing them in 10% (v/v) H₂O₂ solution for 20 min. Afterwards, they were put in aerated 10 mM CaSO₄ solution at 25 °C in the dark overnight. The next day seeds were placed between filter paper soaked in a 4 mM CaSO₄ solution for around three days to germinate and then transferred to the soil-sand substrate gently. Flint and old dent plants were grown in half-cylinder rhizotrons (height 25 cm; diameter 10 cm), for elite dent and hybrids that developed quicker, longer rhizotrons were used (height 48 cm; diameter 10 cm). One seedling was planted per rhizotron. The open part of the half-tubes was covered with a transparent plexiglass observation window and secured with tape. The rhizotrons were arranged in an unblocked randomized design with five biological replicates for each treatment in the climate chamber. Limited by the size of the climate chamber, several growing trials ran from the summer of 2017 to the summer of 2019. The plants were harvested at three weeks after germination. The climate chamber temperature was maintained 25 °C during the day and 20°C at night, air humidity was set to 60%, and light (300–350 μ mol/m² s⁻¹) was from 8 a.m. to 10 p.m. At harvest, the differences in shoot growth of 34 genotypes were easily observed under LP and HP conditions (Figure S4).

2.3 | Harvest and measured traits

Maize P (both shoot and root) and the root traits related to P acquisition were determined. Root traits were classified in root morphological traits and P mobilizing traits (Table S4). Firstly, root exudates were collected from 1 cm subapical root zones of roots by application of strong sorption filter paper (MN156050, Macherey-Nagel, Munich, Germany) and measured as described by Ma et al. (2021). Reverse phase-HPLC in the ion suppression mode at 40°C with isocratic elution (18 mM KH₂PO₄, pH 2.2) on a reversed-phase C-18 column (5 μ m particle size, 290 \times 4.6 mm, GROM-SIL 120 ODS ST) was used to determine organic acids.

After sampling the root exudates, 5 pictures were taken from the root hair zone of each rhizotron under a Stemi 2000-C microscope (Carl Zeiss, Germany). From each picture, 5 root hairs' length was measured using Axio Vision 3.1 software (Carl Zeiss, Germany). The average length of 25 root hairs was taken as one replicate.

After taking photographs of root hairs, roots were carefully lifted out of the soil and shaken to remove bulk soil. Afterwards, the whole root was soaked into 50–100 ml 0.2 mM CaCl₂ for a while to collect rhizosphere soil (Pearse et al., 2007). Rhizosphere soil pH was measured by a pH Meter (SevenCompact pH meter S220, Mettler Toledo), and dent rhizosphere pH was additionally

measured by soil pH meter (HANNA Instrument) directly placed next to the root in the rhizotron. After collecting rhizosphere soil, the roots were carefully washed with deionized water. Acid phosphatase activity at the root surface was assayed using p-nitrophenyl phosphate as the substrate (MP Biomedicals). Two fresh roots were carefully placed into 40 ml of acetate buffer (0.1 mmol/L, pH 5.2) containing 0.5 g·L⁻¹ disodium p-nitrophenyl phosphate. The spectrophotometric assay was carried out at pH 5.2 and 25°C, as recommended by the manufacturer, for one hour. Activity was quantified by comparing the absorption at 405 nm (Hitachi U-3300, Hitachi Ltd. Corporation) to a standard curve of diluted p-nitrophenyl solution and NaOH.

After measuring the acid phosphatase activity, the root systems were stored in 70% (v/v) ethanol. After that, single roots were submerged and separated in a water film on transparent perspex trays and subsequently scanned using a flat-bed scanner at 400 dpi resolution (Epson Expression 1,000 XL). The root length and average root diameter of the scanned images were measured by using the WinRHIZO root analysis system (Reagent Instruments).

The plant materials (shoots, roots and seeds) were oven-dried at 60°C until constant weight for determining dry weight. The dried material was ground to a fine powder. 250 mg shoot dry matter was incubated with HNO₃, H₂O₂, and distilled water in a microwave (MLS Maxi 44) at a maximum of 210°C and 1,400 W for 65 min. This solution was adjusted to 20 ml and filtered through 90-µm mesh filter paper. P was measured spectrophotometrically via orthophosphate determination after the addition of molybdate–vanadate reagent (Gericke & Kurmies, 1952). Based on the P concentration, we calculated P efficiency-related indexes as detailed below.

2.4 | Definitions and abbreviations of phosphorus use efficiency

P use efficiency (PUE) here refers to the ability of plants to produce biomass under particular P conditions (Batten, 1992; Lynch, 1998; Moll et al., 1982). It was calculated by the following formula: PUE (g DW mg⁻¹ P_s) = TDW/P_{substrate}.

where TDW is the total dry weight of the corresponding plants, P_{substrate} is the P amount within the fertilized soil-sand mixture (equal to CAL-P in the soil plus added P per rhizotron), DW is short for dry weight, and P_s is short for P_{substrate}.

PAE refers to the ability of plants to capture P from soils, while PUE is the ability to produce biomass using this acquired P. They were calculated by the following formulas:

$$PAE (\text{mg P mg}^{-1} \text{P}_s) = (P_{\text{content}} - P_{\text{seed}}) / P_{\text{substrate}}$$

with P_{content} is the P amount within the plants, P_{seed} is the P amount within the corresponding seeds. For calculating PAE, we assumed that P_{seed} had been used up at harvest.

$$PUE (\text{g DW mg}^{-1} \text{P}) = \text{TDW} / P_{\text{content}} = 1/[P].$$

where [P] is the plant P concentration with the unit of mg/g DW.

We also calculated the PUE_{w/o seed} representing only the external P acquired from the soil assuming that P_{seed} had been completely

used up by the growing seedlings at harvest. It was calculated by the following formula:

$$PUE_{\text{w/o seed}} (\text{g DW mg}^{-1} \text{P}_s) = [(P_{\text{content}} - P_{\text{seed}}) \times PUE] / P_{\text{substrate}}$$

2.5 | Statistical analyses

All data were analysed by the JMP Pro 15 statistical package (SAS Institute Inc.). In general, the experiment was composed of the two key factors: P level and maize genotypes (or groups from different breeding periods).

Data validity was explored by plotting the response on the x-axis and the genotype or P level on the y-axis. Next, a mixed model with the P level, maize genotype and their interaction as fixed effects was fit, and the assumption of residual normality as well as homogeneity of within-group variances was checked by using diagnostic plots. Data showing irregular or untypical residual plots (residual by predicted plot, residual by row plot and residual quantile plot) were transformed by the logarithm of the raw data (Kozak & Piepho, 2018; Piepho, 2009). To test the time effects of the different experiment runs, random effects were added to the mixed model: Harvesting Date, Harvesting Date × P level, Harvesting Date × Genotype and Harvesting Date × Genotype × P level. Due to the fact that none of the investigated traits showed significant random effects, they were removed from the model. Finally, a mixed model with the P level, maize genotype and P level × maize genotype as fixed effects was used using log-transformed data. Two-way ANOVA was based on this mixed model, followed by Student's *t* test pairwise comparisons at *p* < .05. Mean values were used when fitting linear regressions to PAE, PUE, PUE and root traits with respect to the year of release under LP and HP separately.

3 | RESULTS

3.1 | Seedling biomass and P use efficiencies of groups of flint lines, DH_LRs, dent lines and hybrids

The average shoot dry weight of three-week-old seedlings decreased both in high P (HP) and low P (LP) conditions when grouped according to the year of their release from founder flint lines to the 2nd elite panel and stayed similar thereafter (Figure 1a). The average P concentrations in flint shoots, however, remained unchanged (Figure 1b). Recently produced DH_LRs, which represent 'old' genetics from agronomic systems prior to massive mineral P fertilization, were on average most similar to the second generation of elite flints (Figure 1a,b). The biomass of modern dent lines and hybrids, as expected, was larger than that of flints (Figure 1a). Part of the larger biomass in HP resulted from an apparently more rapid development of some genotypes/groups in HP, namely founder flint lines, dents and the hybrids (Figure 1c). However, at the time of harvest, most DH_LR and flint lines were at the six-leaf stage in HP (average 6.1 ± 0.5). Several genotypes, albeit not all, were slightly delayed in LP (average

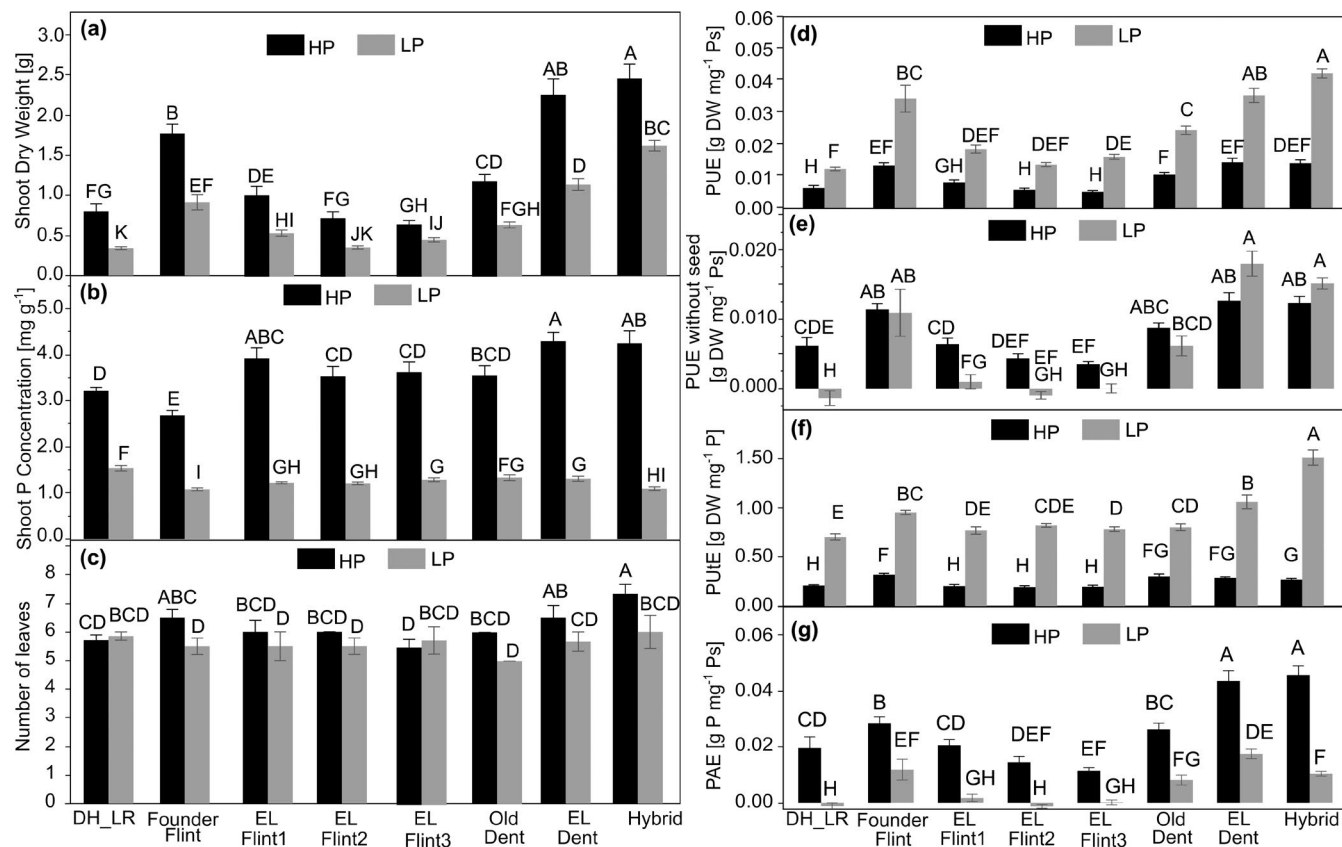


FIGURE 1 Average shoot dry weight, shoot P concentration, leaf numbers and average phosphate efficiencies of different genotype groups. Mean performance \pm SE for shoot dry weight (a), shoot P concentrations (b), leaf number at harvest (c), phosphate use efficiency (d), phosphate use efficiency without seed P (e), phosphate utilization efficiency (f) and phosphate acquisition efficiency (g) were shown. Different letters represent significant differences at $p < .05$ according to the result of Student's *t* test all pairwise comparisons (log-transformed data were used to ensure data normality)

leaf number 5.6 ± 0.5). As expected, elite dents and hybrids developed on average more rapidly, especially in HP, with P415 and P330 \times F047 having already 7–8 leaves at harvest (Figure 1c, Figure S5).

Averaged PUE, PUtE and PAE were calculated for the LP and HP treatments separately and revealed higher PUE and PUtE for each group in LP compared with HP (Figure 1d,f). PAE was always higher under HP. DH_LRs and the elite line panels 1 and 3 were the only groups where the biomass that was produced from external P (excluding seed P) divided by P in the soil (PUE without seed) exhibited significant differences between the P treatments (Figure 1e). Among the groups of flint lines that span 67 years of breeding history, the PUE and PAE declined at each P level from founder lines to the first or second group of elite lines, but did not change since then in the most modern flint elite panel (Figure 1d,g). PUtE was, on average, only higher in founder lines compared with elite lines (Figure 1f).

P efficiency measures of two classical dent lines that are often used in genetic studies (Mo17 and B73) were intermediate with respect to PUE and PAE in both LP and HP. By contrast, modern elite dent lines (and especially three hybrids that were crosses of the studied flints and dents from the same breeding programme) had larger PUtE and simultaneously higher PUE in LP, which was similar to founder flint lines (Figure 1d,f). Their high mean PUE in HP resulted from the high PAE, which was similar in hybrids (Figure 1d,g).

3.2 | Biomass, PUE, PUtE, PAE in the flint heterotic pool and comparison with DH_LRs, dent lines and hybrids

The total seedling dry weight, as well as their root and shoot dry weight, varied, but generally increased with higher P for the 34 genotypes (Figure 2). As expected, most dent seedlings and hybrids had larger biomass than flint seedlings and most of the DH_LRs. A strong growth response to HP compared with LP was found in one DH_LR line (CG1), in several older flints, and some, but not all modern dent lines. The response to P was highly variable among genotypes that were released in the same period (Figure 2). The P levels and the genotypes had significant effects on the shoot and total dry weights, but the root dry weight was little affected by P application. Meanwhile, strong genotype \times P treatment interactions were identified (Table S3).

Individual PUEs and associated efficiencies were first studied in the flint pool. Within these 16 lines, a strong decline of PUE and PAE both in LP and HP was observed, while a moderate decline of PUtE was found significant only at HP level (Figure 3a–c). The decline in PUE was paralleled by a decline in root dry weight in more modern lines, and this was more significant in HP than in LP conditions (Figure 3d). This decline in PUE was not as evident when the dent lines were included in

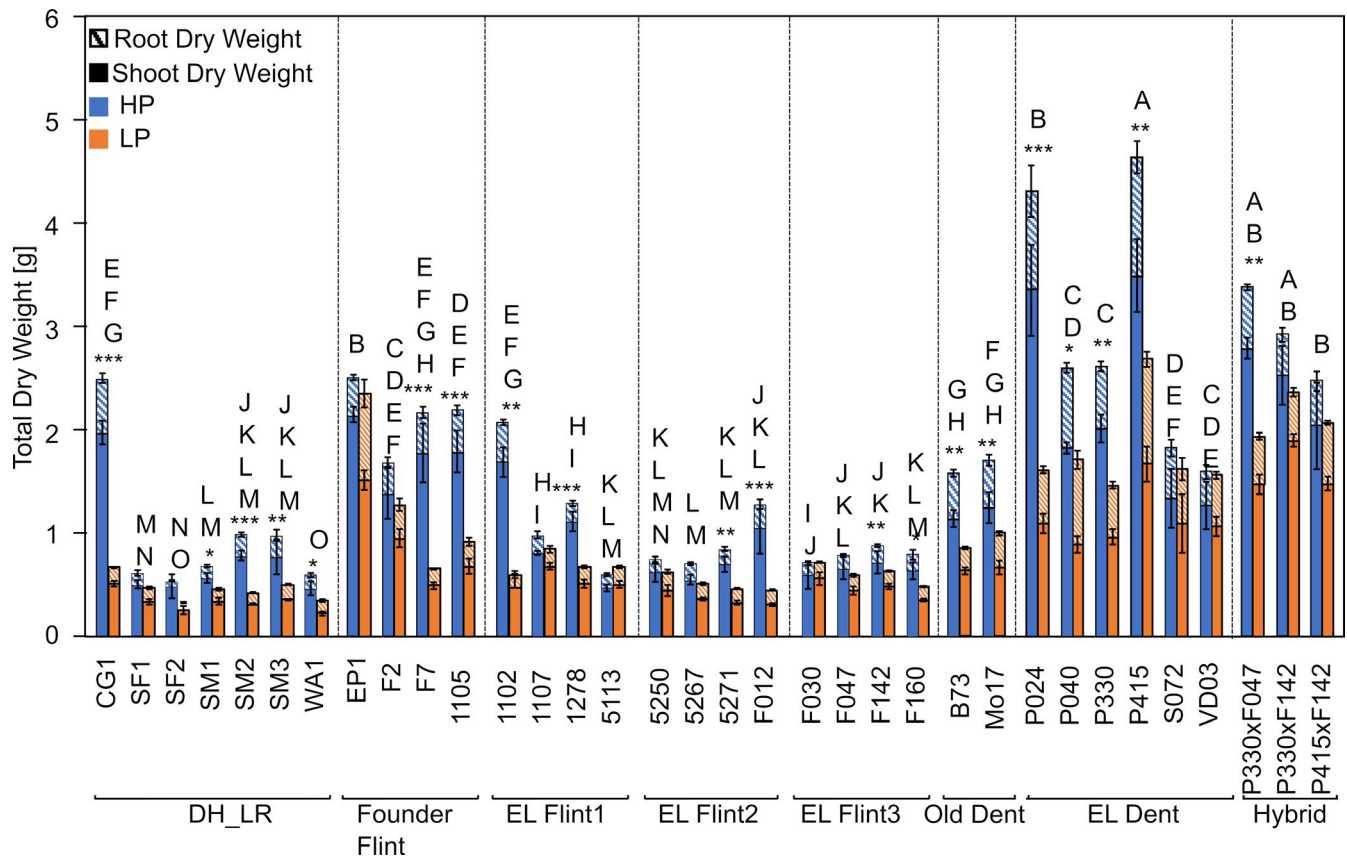


FIGURE 2 Mean performance for total dry weight (TDW) of 34 maize genotypes ($N = 5$) displayed in groups. Significant differences between P treatments and genotype tested based on Student's *t* test pairwise analysis using log-transformed data. Asterisk means differences between P treatment. *, $0.01 < p \leq 0.05$; **, $0.001 < p \leq 0.01$; ***, $p \leq 0.001$. Different letters mean significant difference among genotypes at $p < .05$ [Colour figure can be viewed at wileyonlinelibrary.com]

the graph (Figure S1). PUE and PUE were always higher in LP than in HP (Figure 3a,b), while PAE was higher in HP (Figure 3c). The decline of the dry weight of the roots (and total biomass) of seedlings (Figure 3d) was not seen when landraces, dents and hybrids were included in the graph (Figure S1). The DH_LR lines were overall similar to recent elite flint lines concerning PUE and PAE (Figure 2, Figure S1), despite some variation between individual lines (Figure 2, Figure S1).

We furthermore measured PUE, PUE and PAE in eight dent lines, as well as in three hybrids that were crosses of the studied flint and dent lines (Figure S1). The classical dent lines that are often used in genetic studies (Mo17 and B73) were intermediate with respect to PUE and PAE. The PUE of old dents was similar in to elite flint lines, while modern dent lines and especially hybrids had increased PUE in LP (Figure S1). This elevated PUE caused higher PUEs of dent lines and hybrids in LP. These lines and hybrids also had, on average, increased PUE in HP that was caused by increased PAE (Figure S1).

3.3 | Seed P content and the origin of P in seedlings under LP and HP conditions

The P content per seed substantially varied, between 0.38 and 1.16 mg P seed⁻¹, but without apparent trends related to breeding

history or heterotic group origin (Figure 4a). The seed P concentrations were, however, relatively stable, around 3 mg/g (Figure S2a). Due to efficient remobilization during germination, approximately 1 mg of stored P is sufficient to support seedling growth for around two weeks (Nadeem et al., 2011; White & Veneklaas, 2012). Moreover, the total seedling P content of many genotypes in LP was at seedling harvest in the same range, indicating that several genotypes did not efficiently start to acquire extra P from the soil. In HP, the majority of P in the seedlings invariably came from soil P (Figure 4b). The P from seeds contributed only ~10% of total P in HP in modern dents and hybrids, while the P fraction derived from the seed P was larger in several flint lines. It was highest in F030 (~45%) and the SF2 DH landrace (~50%) (Figure 4b). Interestingly, under LP, the seedlings of more recently released flint lines (1,278, 5,267, 5,271, F012, F142) and some DH landraces (CG1 and SF2) had even less P than was initially stored in the seeds, suggesting that these genotypes lost P during seedling establishment to the environment (Figure 4c). This loss was as large as 50% of seed P in SF2 (DH_LR) (Figure 4c). Modern dent lines, however, accumulated even in LP a large fraction of their total P (~60%) from exogenous P, while the exogenous P fraction was between 37% and 50% in the hybrids in low P. There was only a weak and non-significant trend for an increase in SDW of flints with seed P for

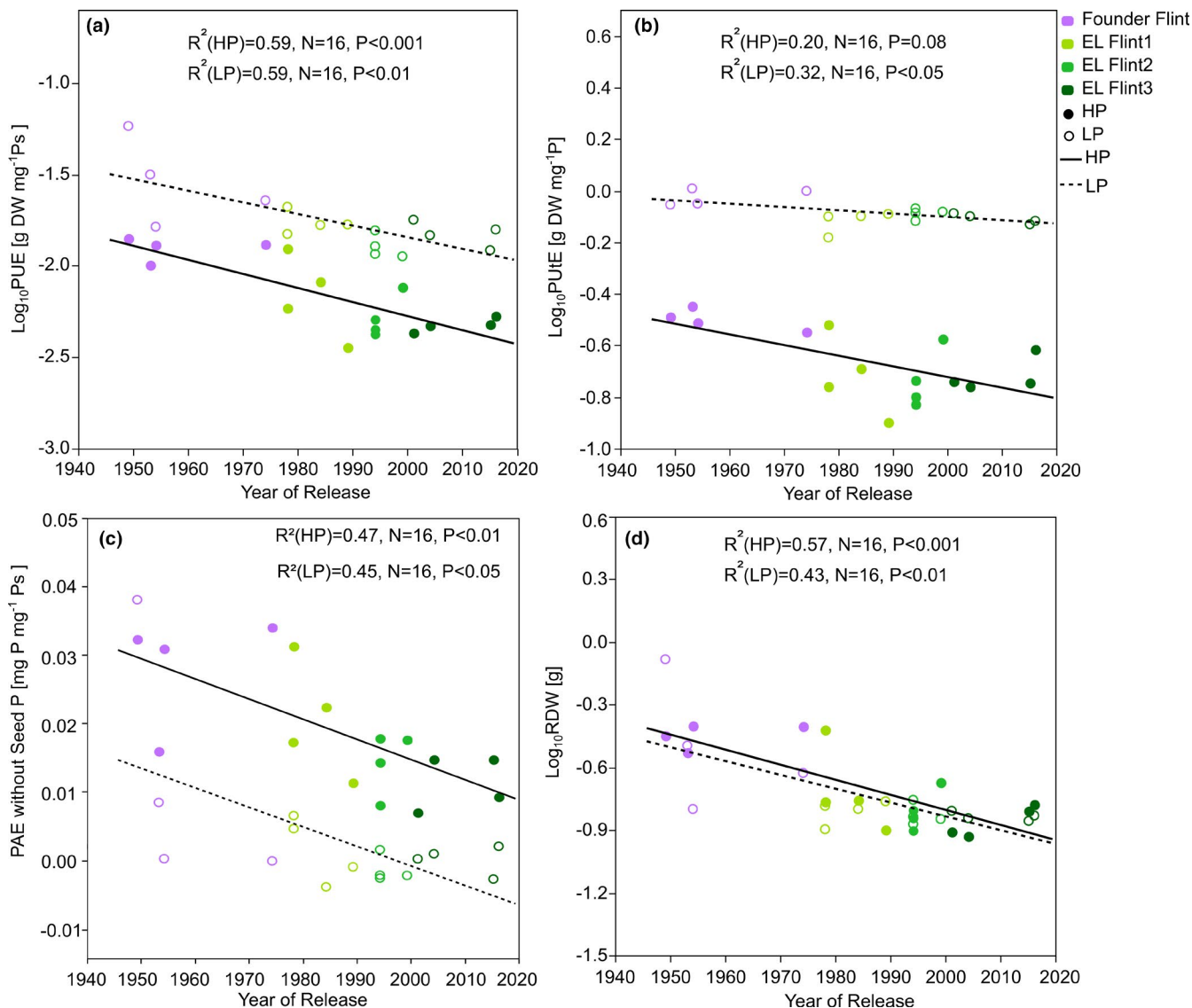


FIGURE 3 Phosphate use efficiency measures of flint material released in different decades. Mean performance for (a) phosphate use efficiency (PUE), (b) phosphate utilization efficiency (PUtE), (c) phosphate acquisition efficiency (PAE) and (d) root dry weight (RDW) of 16 flint maize genotypes plotted versus the year of release of the lines. Regression on the year of release is based on the genotypes in the LP and HP treatment separately. Different colours represent different groups. The open circle represents the mean of each genotype under LP condition; the closed circle represents the mean of each genotype under HP condition. The dashed line stands for the regression curve under LP condition; the solid line stands for the regression curve under HP condition [Colour figure can be viewed at wileyonlinelibrary.com]

LP and HP, respectively (Figure 4d). Seed P did not significantly affect seedling growth at LP or HP, when analysed for all genotypes together (Figure S2b). Likewise, RDW was little affected by seed P (Figure 4e).

There was, however, a robust relationship between the logarithmic TDW of all flints with the P uptake from the soil, both at LP and at HP; this substantial correlation was still observed when DH_LRs, dents and hybrids were included (Figure S2c). Overall, the seed P content was weakly, but significantly ($p < .001$) correlated (0.29) with TDW in LP, whereas the correlation was even higher in HP (0.36, Table S7). In HP, the P concentration was not significantly affected by the seed P, but in LP the P concentration even negatively correlated (-0.43) with seed P, whereas the

P content and seed P content were positively correlated mostly in HP, due to increasing TDW (Table S7).

3.4 | Root traits affected by the breeding progress

We next considered root traits in HP and LP. Root morphological traits included total root length (TRL), specific root length (SRL), average root diameter (RootDiam) and average root hair length (RootHairLen). P mobilizing traits included the amounts of organic acid anions released from root tips (Carboxylates), rhizosphere soil pH (Rhizos-pH) and acid phosphatase activity at the root surface (APase).

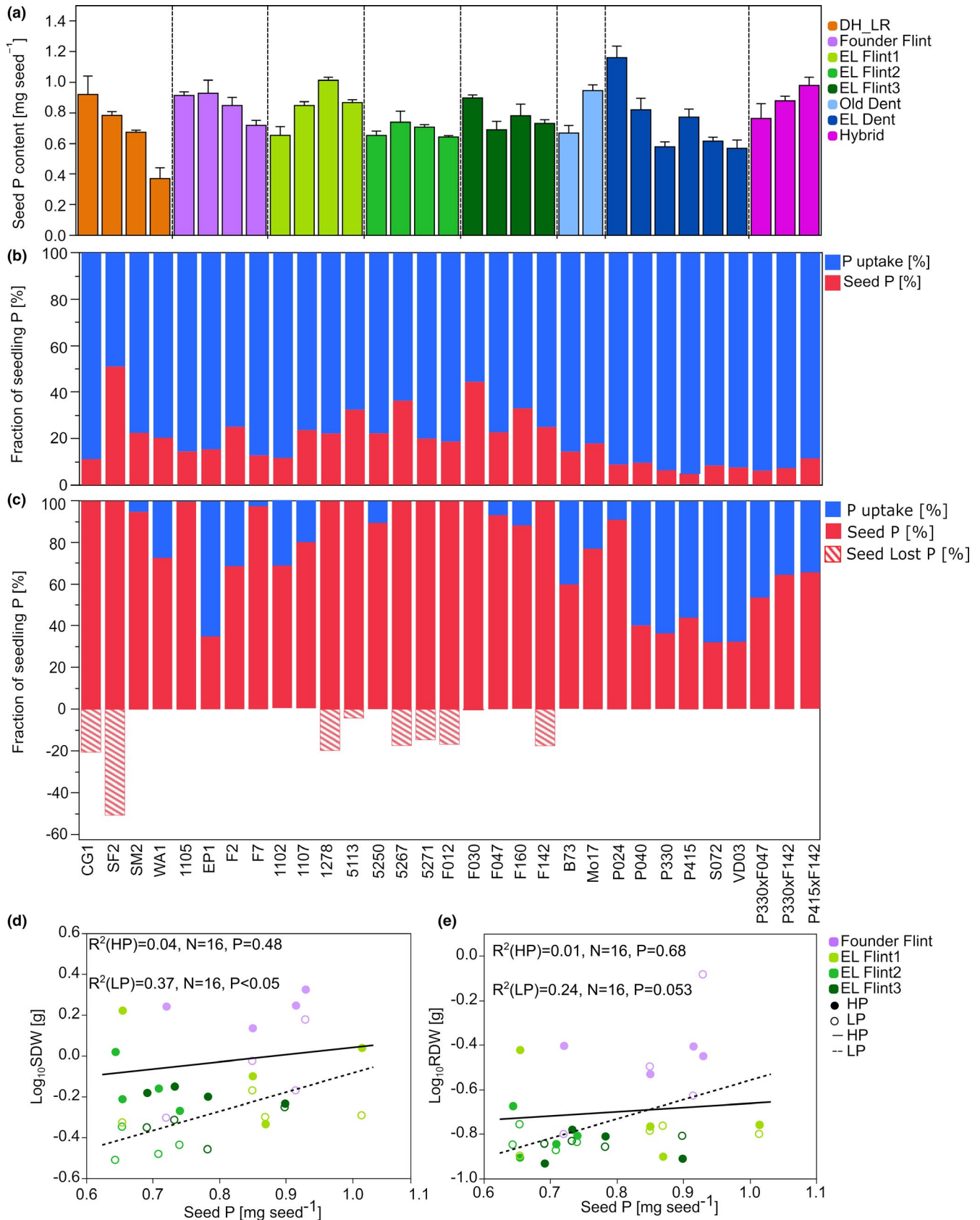


FIGURE 4 Use of seed P. (a) Seed P content in different genotypes. (b, c) The proportional origin of seedling P (red = stored seed P; blue = external P; shaded red: stored seed P lost to the soil) of different lines under high P (b) and low P conditions (c). (d) Relation between shoot dry weight (SDW) and seed P in the LP and HP treatments for 16 flints. (e) Relation between root dry weight (RDW) and seed P in the LP and HP treatments for 16 flints [Colour figure can be viewed at wileyonlinelibrary.com]

There was a significant effect of genotypes on TRL and SRL, but there was no P treatment effect for these two measures (Tables S5). Genotypes, treatments and their interaction significantly influenced other root morphological traits, such as RootDiam, root-to-shoot ratio (Root/Shoot) and RootHairLen. P mobilizing traits, which include Carboxylates, Rhizos-pH and APase, were also significantly affected by the genotype, treatment and the interaction (Table S5).

When separately evaluated for the flint lines in LP, morphological traits, such as Root/Shoot ratio, TRL, SRL and RootDiam (Figure 5a-d), but also P mobilizing traits, such as APase and Carboxylates, were little affected by the breeding progress (Figure 5e-h). By contrast, Rhizo-pH and RootHairLen in LP declined in more recent released genotypes (Figure 5e-h). Root morphological and mobilizing traits were also measured in the other lines, and their measures are displayed in the same diagrams (Figure 5). Notable is the general decline of TRL with the breeding progress. This was comparable in modern dent lines and hybrids, but note the relatively thick RootDiam and increased Carboxylates in all dent lines and hybrids (Figure 5). DH_LRs were neither outstanding in morphological nor mobilizing traits and were similar to the moderately modern flints (Figure 5).

Although the internal remobilization of stored P was the crucial P source in LP at the investigated juvenile stage, all root traits except APase were significantly correlated with PAE in LP (Table 1). Overall, correlations of efficiency measures were more significant in LP than in HP. TRL and SRL were always associated with PUE and PAE, regardless of the P treatments. In LP, all root traits except APase and Root/Shoot were correlated considerably to PUE, and all root traits except APase were correlated with PAE. Furthermore, in LP, SRL, RootDiam and Rhizos-pH were correlated with PUTe, while APase was correlated with PUE and PAE in HP (Table 1). SRL was inversely correlated with all of the measured root functional traits, except TRL in LP. Most root functional traits inversely correlated with SRL in HP (Table 2). In LP, the strongest correlations for SRL were found with RootDiam ($r = -.70$), TRL ($r = .33$) and Root/Shoot ($r = -.35$), which is expected from the definitions of these traits. Within the HP treatment, correlations between SRL and TRL, Root/Shoot, RootDiam and APase were apparent. RootHairLen was significantly associated with all root traits, except SRL, Root/Shoot and APase in LP. Apart from SRL, a significantly negative correlation between two root traits under LP was only found between RootDiam and RootHairLen. In HP, TRL was negatively correlated with RootHairLen. APase showed no correlation with other root traits in LP and was therefore the least relevant trait for P use among all the eight functional traits in LP, although under HP, APase was found correlated with SRL (Table 2).

Moreover, Principal Component Analysis (PCA) with PUE, PAE and PUTe, as well as all eight root functional traits, revealed distinct patterns for founder and elite flints. The first two components together explained more than 58% of the total variation in both HP and LP treatments. Mostly in LP, founder flint genotypes were separated from the other lines (Figure 6). PUE and RootHairLen were mainly represented by PCA1, but interestingly, RootHairLen contributed oppositely in LP and HP (Figure 6).

4 | DISCUSSION

The application of mineral P fertilizers increases plant-available P in the soil and improves crop growth and yield, but its success depends on many different factors such as type of fertilizer, application form, the timing of fertilization, weather conditions, various soil properties and plant factors (Holford, 1997; Shen et al., 2011). As P is a finite resource, breeding of major crops with improved P efficiency may have large effects on the sustainability of the agroecosystems (Ludewig et al., 2019). Efforts in breeding tropical maize for P-deficient, acid soils with high loads of toxic aluminium have already identified candidate loci for important root traits (Azevedo et al., 2015), an example of positive breeding impact on root traits.

4.1 | The decline of PUE in flint lines along with the breeding history

In this study, there was substantial variation in maize seedling growth of elite varieties under low and high P conditions. The biomass of modern flint seedlings decreased compared with founder flints, which was inconsistent with our expectations, as modern varieties are selected for higher final yields (Figures 1a and 2). In elite flints released from the 1950s to 2010s, PUE, PUTe and PAE decreased dramatically under both low P and high P (Figure 3a-c). Surprisingly, most DH_LRs performed similar to moderately modern elite flints (Figures 1a and 2). Apparently, there is no increase in P utilization accompanied with the breeding process in flints, but there are different trends in biomass and phosphorus utilization in dents and hybrids. The decline of PUE and PAE of elite flints was accompanied with smaller seedling root biomass despite the breeding progress (Figure 3), while Root/Shoot did not considerably change (Figure 5a). This may imply for flints that investment into the roots may be of less importance in well-managed soils with all nutrients amply available, as in typical breeding scenarios. The reduction in RootHairLen may explain part of the decrease in PAE under LP within the flint pool, as PAE and RootHairLen are positively correlated under LP (Table 1 and Figure 5h). In addition, elite flint lines were apparently on average not capable to acquire external P under LP conditions, in contrast to founder flint lines (Figure 4c).

In the conditions tested, most dent elite varieties performed superior than the flints. Those selected in the same public breeding programme released from the 1990s to 2010s generally had higher biomass, PUE, PUTe and PAE. These values were comparable to some flint founder lines (Figures 1d-g and 3a-c), which directly verified the hypothesis that increased biomass of modern elite varieties is associated with increased PUE, PAE and/or PUTe compared with old genotypes and landraces. PAE of all the genotypes was higher in HP than in LP, pointing to the importance of seed P under LP and probably reflecting the selection process with high P fertilization (Figure 1g). It is noteworthy that the founder flint EP1 was superior to most other flint lines in LP and similar to the modern elite dent P415. Moreover, EP1 was the only flint line that exhibited a similar P uptake as the dent lines,

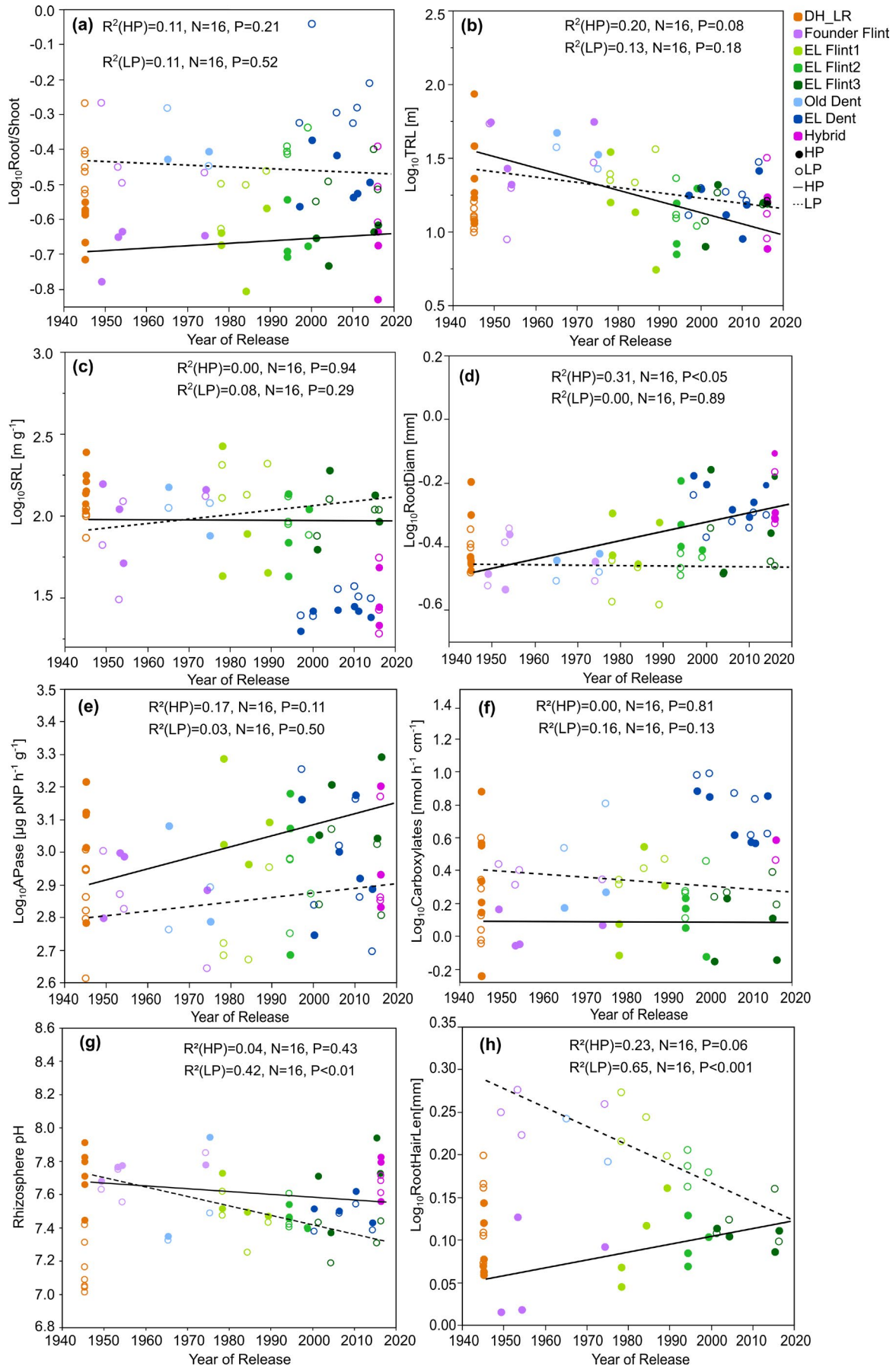


FIGURE 5 Root morphological and rhizosphere traits of genotypes and relation to their year of release. Mean performance of (a) root-to-shoot ratio, (b) total root length, (c) specific root length, (d) average root diameter, (e) acid phosphatase activity, (f) Carboxylates, (g) rhizosphere pH and (h) root hair length of 34 maize genotypes plotted versus the year of release of the lines and hybrids. Different colours represent different groups. The open circles represent the mean of each genotype under LP condition; the closed circles represent the mean of each genotype under HP condition. The linear regressions were separated for LP (dashed line) and HP (solid line) treatments and only included flint lines [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Pearson's correlation coefficients for P efficiency and root functional traits with log-transformed and non-transformed data (PAE) for 34 maize genotypes in response to high soil phosphorus availability and low soil phosphorus availability

Treatment	P efficiency	TRL	SRL	Root/Shoot	APase	RootDiam	Rhizos-pH	Carboxylates	RootHairLen
HP	PUE	0.50***	-0.38**	0.17	-0.25*	0.15	-0.05	0.06	-0.19
	PAE	0.29*	-0.48***	0.15	-0.27*	0.27*	-0.08	0.27*	-0.22
	PUtE	0.34***	-0.17	-0.05	-0.1	-0.09	0.16	-0.22	-0.07
LP	PUE	0.39***	-0.67***	0.18	0.04	0.41***	0.4***	0.45***	0.45***
	PAE	0.31**	-0.54***	0.44***	0.09	0.20*	0.28**	0.42***	0.33***
	PUtE	0.17	-0.47***	-0.03	0.03	0.41***	0.26**	0.17	0.15

Note: The data for APase are without SM3 ($n = 33$); Rhizos-pH without P024 & P330 ($n = 32$); RootHairLen without SM3, dents & hybrids ($n = 22$); Carboxylates without P330 × F142 & P415 × F142 ($n = 32$). Root trait abbreviations: TRL, total root length; SRL, specific root length; Root/Shoot, root-to-shoot ratio; APase, acid phosphatase activity on the root surface; RootDiam, average diameter; Rhizos-pH, rhizosphere soil solution pH; RootHairLen, average root hair length; Carboxylates, the amounts of carboxylates in the rhizosheath. Significant correlations are in bold: *, $0.01 \leq p < .05$; **, $0.001 \leq p < .01$; ***, $p < .001$.

TABLE 2 Pearson's correlation coefficients for pairwise root functional traits with log-transformed data for 34 maize genotypes in response to high soil phosphorus availability (upper-right diagonal) and low soil phosphorus availability (low-left diagonal)

	TRL	SRL	Root/Shoot	APase	RootDiam	Rhizos-pH	Carboxylates	RootHairLen
TRL		0.52***	0.12	-0.07	-0.24	-0.01	-0.05	-0.32**
SRL	0.33***		-0.35**	0.24*	-0.46***	0.07	-0.20	-0.16
Root/Shoot	0.22*	-0.35***		-0.19	0.16	-0.10	0.17	-0.07
APase	-0.10	-0.18	0.16		0.02	-0.05	-0.14	0.18
RootDiam	0.35***	-0.70***	0.11	-0.07		-0.22	0.15	0.15
Rhizos-pH	0.12	-0.31**	0.03	0.06	0.05		-0.09	-0.16
Carboxylates	0.18	-0.43***	0.36***	0.07	0.21*	0.19		-0.08
RootHairLen	0.37***	-0.03	0.09	-0.20	-0.36***	0.48***	0.22*	

Note: The data for APase are without SM3 ($n = 33$); Rhizos-pH without P024 & P330 ($n = 32$); RootHairLen without SM3, dents & hybrids ($n = 22$); Carboxylates without P330 × F142 & P415 × F142 ($n = 32$). Root trait abbreviations: TRL, total root length; SRL, specific root length; Root/Shoot, root-to-shoot ratio; APase, acid phosphatase activity on the root surface; RootDiam, average diameter; Rhizos-pH, rhizosphere soil solution pH; RootHairLen, average root hair length; Carboxylates, the amounts of carboxylates in the rhizosheath. Significant correlations are in bold: *, $0.01 \leq p < .05$; **, $0.001 \leq p < .01$; ***, $p < .001$.

of nearly 70% in LP. P415 was more responding to P fertilization than EP1 and doubled its biomass, while EP1 did not differ in TDW under LP and HP (Figure 2). Both lines were apparently P efficient in LP, but only P415 showed additional P responsiveness.

4.2 | Different P efficiency and root traits between flint and dent panels

Many examples have shown that P efficiency mechanisms are different from one genotype to another within a given plant species

(Bayuelo-Jiménez et al., 2011; Colombi et al., 2019; Li et al., 2019; Ozturk et al., 2005; Tang et al., 2020; York et al., 2015). There are substantial variability and distinct genetic architectures in dent and flint maize diversity panels (Cartea et al., 1999; Messmer et al., 1992; Rincent et al., 2014), which is consistent with different P efficiency trends of the two panels. Due to different geographic separation and contrasting environmental conditions, phenotypic differences between these two germplasm pools are expected (Brown & Anderson, 1947; Unterseer et al., 2016). Much of the large variation in seedling biomass between these two germplasm pools was probably due to different P remobilization from seeds, and a

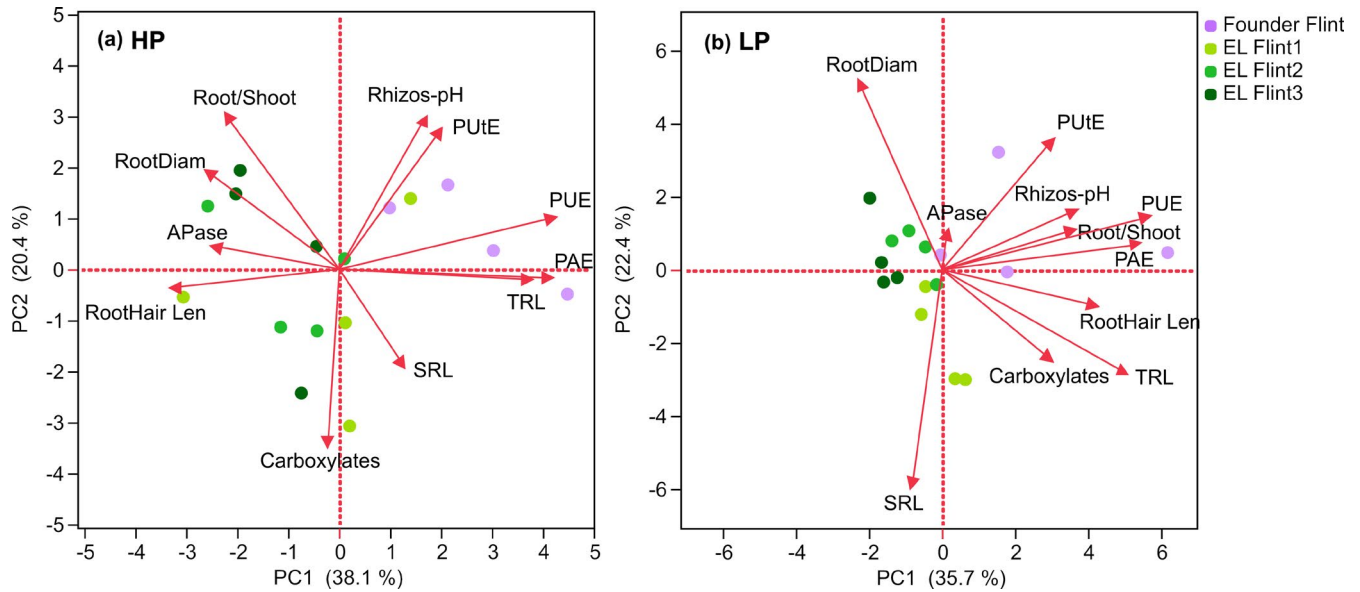


FIGURE 6 Principal component analysis (PCA) of root functional traits and phosphate efficiency for 16 genotypes in different classes with high (a) and low (b) soil phosphorus availability. Root trait abbreviations in figures: TRL, total length of the whole root system; SRL, length per dry mass of the whole root system; RootDiam, average diameter of the whole root system; Carboxylates, the total amount of organic acid anions collected by filter papers on the surface of root tips; APase, acid phosphatase activity in the rhizosphere. Rhizos-pH, the pH of the rhizosphere soil solution or pH in the rhizosphere; Root/Shoot, the ratio of root dry weight to shoot dry weight; PUE, phosphorus use efficiency; PAE, phosphorus acquisition efficiency; PUE, phosphorus utilization efficiency [Colour figure can be viewed at wileyonlinelibrary.com]

P responsive traits	Founder flint		Elite flint (EL3)		P responsive traits
	LP	HP	HP	LP	
Shoot dry weight HP > LP					Shoot dry weight HP > LP
Total root length HP > LP					Apase activity HP > LP
Root diameter HP < LP					Root diameter HP > LP
Root to shoot ratio HP < LP					Root to shoot ratio HP < LP
Specific root length HP > LP					Rhizosphere pH HP > LP
Carboxylates HP < LP					Carboxylates HP < LP
Breeding process					
Increase of APase activity	Increase of average root diameter (HP) Decrease of average diameter (LP) Increase of specific root length (LP)				Decrease of biomass Decrease of Total root length

FIGURE 7 Schematic model summarizing the major observations of traits contributing to PUE, PAE and PUE of founder flint and most modern flint EL3. Schematic plant appearance for LP and HP is shown [Colour figure can be viewed at wileyonlinelibrary.com]

delayed switch to exogenous P acquisition in modern flints in LP. Especially in LP, seedlings of several genotypes (especially some in the Elite Flint2 and Elite Flint3 groups) even contained less P than

originally stored in the seed (Figure 4c). This suggests that during germination these were not able to utilize the remobilized seed P and failed to import it into the seedling, resulting in a net seed P

loss. Massive losses of macronutrients and micro-nutrients during maize germination have been reported and can be prevented by silicon treatments (Moradtab et al., 2018). In juvenile maize seedlings, re-uptake of transiently lost seed P from internal sources appears thus a major function of the root system. Previous research showed that the measurable P uptake by maize roots begins with a delay of around five days after germination, but in the variety of that study, the P uptake rate was independent of the initial seed P content and was little affected by external P (Nadeem et al., 2011; White & Veneklaas, 2012). For the different genetic backgrounds analysed here, there was a strong correlation of total dry mass with exogenous P taken up, especially in HP, but not with the seed P content (Table S7). Some dent elite lines (and hybrids) apparently switched earlier or more robustly to acquisition from exogenous P pools, so that they had already acquired substantial external P at the time of harvest (Figure 4). These consequently produced more seedling biomass.

Several root traits differed between flint and dent panels, and between different lines. On average, dent lines had increased RootDiam and released more Carboxylates, while TRL decreased in modern dents compared with old dents in HP (Figure 5). However, comparisons of the elite dents with these old dent lines must be taken with care, as their genetic origin is different, and also the sample size within these groups differed. In addition to the genetic differences, the temperature setting of the climate chamber may have been more favourable for the dent lines compared with the flints, the latter are cold tolerant and this may explain part of the differences between the flint and dent lines. While the different morphological characteristics between the flint and dent panels can be beneficial in view of hybrid breeding and the associated heterosis effect, it is important to note that beneficial traits in hybrids were more similar to dents, but were overall very little related to their flint or dent parents. The comparison of each hybrid with its parents indicated that all PUE traits were a complex additive combination of their parents that was dominated by the more efficient dent parent and was often, but not always, superior to its parents.

4.3 | Trade-offs of root traits related to PUE under LP

Several root traits were found to be associated with PUE in the breeding progress, but this was different in LP and HP. In HP, only TRL was positively correlated with PUE, implying that breeding with ample P resulted in an unintentional selection for TRL. In LP, TRL, RootDiam, Rhizos-pH, Carboxylates and RootHairLen were positively correlated with PUE, suggesting that genotypes bred under ample P remained some relationships with root traits important for limited P (Table 1). Moreover, RootHairLen and Carboxylates exhibited the highest positive contribution to PUE in LP (Table 1). Within the flint pool, several root traits were associated with the decrease in PUE in the breeding process: Rhizos-pH and RootHairLen in LP, and to a lesser extent RootDiam in HP

(Figure 5). Founder flints had a weaker ability to acidify the rhizosphere and grew longer root hairs in limiting P conditions, which verified the hypothesis that modern elite genotypes lost beneficial root-related traits under limited P supply, because of selection under ample P. Interestingly, RootHairLen was the only root functional trait where Pearson's correlation of PAE significantly shifted from a negative to positive value with different P treatments (Table 1).

A wide variation and co-variation of key root and rhizosphere traits was found. This creates multiple P acquisition strategies that may be similarly efficient in an ecosystem (Brundrett & Tederloo, 2018; Lambers et al., 2018; Zemunik et al., 2015). Previous studies showed that in controlled experimental conditions, different plant species showed different P acquisition strategies with different root and rhizosphere trait trade-offs. Maize with its fibrous root system invests relatively little into rhizosphere traits (Lyu et al., 2016; Wen et al., 2019). In our study, only TRL and SRL (morphological traits) were not significantly affected by P treatment, and all the traits (morphological traits and rhizosphere traits) showed variation among genotypes (Table S5). Meanwhile, the effect of the breeding process on TRL and SRL was not different among the P treatments (Figure 5b,c). A high co-variation of root functional traits was found in our 34 genotypes. In low P, the correlation between SRL and investigated root traits except TRL was negative (Table 2). The negative correlations between SRL and the RootDiam were already reported in other studies dealing with P responsive root traits within maize seedlings (Zhu & Lynch, 2004). This is due to its definition ($SRL = TRL/RDW$). Postma and Lynch (2011) claimed that with low P supply, maize roots increased cortical aerenchyma, which may lead to a decrease in root biomass and an increase in SRL, without changes in root diameter. RootHairLen was positively correlated with TRL, Carboxylates and Rhizos-pH under LP, but negatively correlated with RootDiam (Table 2), showing that in LP, genotypes with longer root hairs released more organic acids and had more fine roots. Many studies demonstrated the importance of fine roots in P uptake (Lynch, 2011). Therefore, it is assumed that the positive contribution of RootDiam to PUE is mainly based on the high correlation between RootDiam and the biomass. This may also explain the correlation between Root/Shoot and PAE in LP compared with HP.

As we have mentioned, TRL was important in both LP and HP for PUE; RootDiam, Rhizos-pH, Carboxylates and RootHairLen were more important in LP for PUE. In HP, TRL was negatively correlated with RootHairLen ($r = -.32$), and in LP, TRL was positively correlated with RootHairLen ($r = .37$) (Table 2). Moreover, the correlation between PUE and TRL decreased from 0.50 in HP to 0.39 in LP. The correlation between PUE and RootHairLen increased from -0.19 to 0.45 (Table 1). Altogether, this indicates trade-offs among the root traits, which verified our ultimate hypothesis. The variation and co-variation still existed when only considering exclusively the flint pool. In HP, there was a large variation of all these eight root functional traits; in LP, there was co-variation among TRL, RootHairLen,

Root/Shoot, Rhizos-pH and Carboxylates (Figure 6). Trade-offs between TRL and RootHairLen are found in the flint pool (Figure 6), which confirmed that breeding selection can have negative impacts under different environmental conditions.

Meanwhile, maize genotypes released in different breeding periods showed different P acquisition strategies. A schematic summary of the different strategies of old and modern elite flint lines to acquire and utilize P efficiently in LP and HP is given in Figure 7 and for dent lines in Figure S3. Dent lines exhibited the lowest SRL values, which means they produced thicker roots than flint lines. The high RootDiam within the dent lines is indicative of a different root system with long, thick seminal and crown roots (Figure 5h). On the other hand, the production of dent root biomass required less P (Table S6). Modern dent lines exhibited the highest PUE and had the highest average root diameter. The weak Root/Shoot and RootDiam correlation and that with several other traits that were different in LP and HP, potentially indicates a trade-off for acclimation to contrasting P conditions, as the increased contribution of one trait resulted in the decreased contribution of another trait (Table 2). The negative trend for PUE in juvenile flints was corroborated in another soil (Li et al., 2021), but as only young roots were considered here, field trials with the investigated genotypes are required to confirm the importance of juvenile PUE traits for adult plants and for final grain yield and grain PUE. Such experiments should also consider other traits that are important for P efficiency in adult plants, such as the presence of cortical root aerenchyma (Postma & Lynch, 2011) or the association with mycorrhiza (Li et al., 2021).

5 | CONCLUSION

When evaluating root traits associated with high P efficiency, seed P should also be considered. A decline in PUE in modern maize elite flint material was associated with smaller roots, shorter root hairs and less rhizosphere acidification, while high PUE was identified in elite dents and hybrids from the same breeding programme. Some pre-selected doubled haploid landraces were similar to modern elite varieties. Although the relationships of root traits and their contribution to optimal PUE, PuE and PAE in juvenile maize flint and dent pools need to be further verified in different soils, growth conditions and in mature plants, our study may help to focus on improving P use in maize agroecosystems to contribute to more sustainable agriculture.

ACKNOWLEDGEMENTS

This study was supported by the German Research Foundation (DFG)—328017493/GRK 2366 (International Research Training Group 'Adaptation of maize-based food-feed-energy systems to limited phosphate resources'). We gratefully acknowledge the China Scholarship Council (201606350143) for supporting X. L. Open access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION

We thank H. Ochott for help with nutrient analyses and Prof. Dr. Günter Neumann for discussion. X. L., M. M., A. M. and U. L. conceived the research. X. L., M. M. and U.L. designed the research. X. L. and M. M. conducted the experiments and analysed the data with help of H.-P. P. The manuscript was written by X. L. and M.M., and U.L. edited the manuscript. All authors read and approved the manuscript.

DATA AVAILABILITY STATEMENT

Data are available in the supplement.

ORCID

Xuelian Li  <https://orcid.org/0000-0002-0746-0048>

Uwe Ludewig  <https://orcid.org/0000-0001-5456-1055>

REFERENCES

- Akhtar, M. S., Oki, Y., & Adachi, T. (2008). Genetic variability in phosphorus acquisition and utilization efficiency from sparingly soluble P-sources by *Brassica* cultivars under P-stress environment. *Journal of Agronomy and Crop Science*, *194*, 380–392. <https://doi.org/10.1111/j.1439-037X.2008.00326.x>
- Azevedo, G. C., Cheavegatti-Gianotto, A., Negri, B. F., Hufnagel, B., e Silva, L. D. C., Magalhaes, J. V., Garcia, A. A. F., Lana, U. G. P., de Sousa, S. M., & Guimaraes, C. T. (2015). Multiple interval QTL mapping and searching for *PSTOL1* homologs associated with root morphology, biomass accumulation and phosphorus content in maize seedlings under low-P. *BMC Plant Biology*, *15*, 172. <https://doi.org/10.1186/s12870-015-0561-y>
- Barry, D. A. J., & Miller, M. H. (1989). Phosphorus nutritional requirement of maize seedlings form maximum yield. *Agronomy Journal*, *81*, 95–99. <https://doi.org/10.2134/AGRONJ1989.00021962008100010017X>
- Batten, G. D. (1992). A review of phosphorus efficiency in wheat. *Plant and Soil*, *146*, 163–168. <https://doi.org/10.1007/BF00012009>
- Bayuelo-Jiménez, J. S., Gallardo-Valdéz, M., Pérez-Decelis, V. A., Magdaleno-Armas, L., Ochoa, I., Lynch, J., & Lynch, J. P. (2011). Genotypic variation for root traits of maize (*Zea mays* L.) from the Purhepecha Plateau under contrasting phosphorus availability. *Field Crops Research*, *121*, 350–362. <https://doi.org/10.1016/j.fcr.2011.01.001>
- Bielecki, R. L. (1973). Phosphate pools, phosphate transport, and phosphate availability. *Annual Review of Plant Physiology*, *24*, 225–252. <https://doi.org/10.1146/annurev.pp.24.060173.001301>
- Brown, W. L., & Anderson, E. (1947). The northern flint corns. *Annals of the Missouri Botanical Garden*, *34*, 1–29. <https://doi.org/10.2307/2394437>
- Brundrett, M. C., & Tedersoo, L. (2018). Evolutionary history of mycorrhizal symbioses and global host plant diversity. *The New Phytologist*, *220*, 1108–1115. <https://doi.org/10.1111/nph.14976>
- Cartea, M. E., Revilla, P., Butrón, A., Malvar, R. A., & Ordás, A. (1999). Do second cycle maize inbreds preserve the European flint heterotic group? *Crop Science*, *39*, 1060–1064. <https://doi.org/10.2135/cropsci1999.0011183X003900040017x>
- Colombi, T., Herrmann, A. M., Vallenback, P., & Keller, T. (2019). Cortical cell diameter is key to energy costs of root growth in wheat. *Plant Physiology*, *180*, 2049–2060. <https://doi.org/10.1104/pp.19.00262>
- Erel, R., Bérard, A., Capowiez, L., Doussan, C., Arnal, D., Souche, G., & Hinsinger, P. (2017). Soil type determines how root and rhizosphere traits relate to phosphorus acquisition in field-grown maize genotypes. *Plant and Soil*, *412*, 115–132. <https://doi.org/10.1007/s11104-016-3127-3>

- Gericke, S., & Kurmies, B. (1952). Colorimetrische Bestimmung der Phosphorsäure mit Vanadat-Molybdat: VM-Methode: Springer-Verlag Berlin Heidelberg. <https://doi.org/10.1007/978-3-662-11336-3>
- Haling, R. E., Brown, L. K., Stefanski, A., Kidd, D. R., Ryan, M. H., Sandral, G. A., & Simpson, R. J. (2018). Differences in nutrient foraging among *Trifolium subterraneum* cultivars deliver improved P-acquisition efficiency. *Plant and Soil*, 424, 539–554. <https://doi.org/10.1007/s11110-017-3511-7>
- Hinsinger, P. (2001). Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: A review. *Plant and Soil*, 237, 173–195. <https://doi.org/10.1023/A:1013351617532>
- Holford, I. C. R. (1997). Soil phosphorus: Its measurement, and its uptake by plants. *Australian Journal of Soil Research*, 35, 227–239. <https://doi.org/10.1071/S96047>
- Hölker, A. C., Schipprack, W., Utz, H. F., Molenaar, W. S., & Melchinger, A. E. (2019). Progress for testcross performance within the flint heterotic pool of a public maize breeding program since the onset of hybrid breeding. *Euphytica*, 215(3), Article 50. doi: 10.1007/s10681-019-2370-0
- Irshad, U., Brauman, A., Villenave, C., & Plassard, C. (2012). Phosphorus acquisition from phytate depends on efficient bacterial grazing, irrespective of the mycorrhizal status of *Pinus pinaster*. *Plant and Soil*, 358, 155–168. <https://doi.org/10.1007/s11104-012-1161-3>
- Kozak, M., & Piepho, H.-P. (2018). What's normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. *Journal of Agronomy and Crop Science*, 204, 86–98. <https://doi.org/10.1111/jac.12220>
- Lambers, H., Albornoz, F., Kotula, L., Laliberté, E., Ranathunge, K., Teste, F. P., & Zemunik, G. (2018). How belowground interactions contribute to the coexistence of mycorrhizal and non-mycorrhizal species in severely phosphorus-impooverished hyperdiverse ecosystems. *Plant and Soil*, 424, 11–33. <https://doi.org/10.1007/s11104-017-3427-2>
- Li, H., Zhang, D., Wang, X., Li, H., Rengel, Z., & Shen, J. (2019). Competition between *Zea mays* genotypes with different root morphological and physiological traits is dependent on phosphorus forms and supply patterns. *Plant and Soil*, 434, 125–137. <https://doi.org/10.1007/s11104-018-3616-7>
- Li X, Quan X, Mang M, Neumann G, Melchinger A., & Ludewig U. (2021). Flint maize root mycorrhization and organic acid exudates under phosphorus deficiency: Trends in breeding lines and doubled haploid lines from landraces. *Journal of Plant Nutrition and Soil Science*. <https://doi.org/10.1002/jpln.202000471>
- Liu, Z., Gao, K., Shan, S., Gu, R., Wang, Z., Craft, E. J., & Chen, F. (2017). Comparative analysis of root traits and the associated QTLs for maize seedlings grown in paper roll, hydroponics and vermiculite culture system. *Frontiers in Plant Science*, 8, Article 436. <https://doi.org/10.3389/fpls.2017.00436>
- Ludewig, U., Yuan, L., & Neumann, G. (2019). Improving the efficiency and effectiveness of global phosphorus use: Focus on root and rhizosphere levels in the agronomic system. *Frontiers of Agricultural Science and Engineering*, 6, 357–365. <https://doi.org/10.15302/J-FASE-2019275>
- Lynch, J. (1998). The role of nutrient-efficient crops in modern agriculture. *Journal of Crop Production*, 1, 241–264. https://doi.org/10.1300/J144v01n02_10
- Lynch, J. (2011). Root phenes for enhanced soil exploration and phosphorus acquisition: Tools for future crops. *Plant Physiology*, 156, 1041–1049. <https://doi.org/10.1104/pp.111.175414>
- Lyu, Y., Tang, H., Li, H., Zhang, F., Rengel, Z., Whalley, W. R., & Shen, J. (2016). Major crop species show differential balance between root morphological and physiological responses to variable phosphorus supply. *Frontiers in Plant Science*, 7, Article 1939. doi: 10.3389/fpls.2016.01939
- Ma, X., Li, X., & Ludewig, U. (2021). Arbuscular mycorrhizal colonization outcompetes root hairs in maize under low phosphorus availability. *Annals of Botany*, 127, 155–166. <https://doi.org/10.1093/aob/mcaa159>
- Ma, Z., Guo, D., Xu, X., Lu, M., Bardgett, R. D., Eissenstat, D. M., & Hedin, L. O. (2018). Evolutionary history resolves global organization of root functional traits. *Nature*, 555, 94–97. <https://doi.org/10.1038/nature25783>
- Manske, G., Ortiz-Monasterio, J. I., van Ginkel, M., González, R. M., Fischer, R. A., Rajaram, S., & Vlek, P. (2001). Importance of P uptake efficiency versus P utilization for wheat yield in acid and calcareous soils in Mexico. *European Journal of Agronomy*, 14, 261–274. [https://doi.org/10.1016/S1161-0301\(00\)00099-X](https://doi.org/10.1016/S1161-0301(00)00099-X)
- Messmer, M. M., Melchinger, A. E., Boppenmaier, J., Herrmann, R. G., & Brunklaus-Jung, E. (1992). Rflp analyses of early-maturing European maize germ plasm: I. Genetic diversity among flint and dent inbreds. *Theoretical and Applied Genetics*, 83, 1003–1012. <https://doi.org/10.1007/BF00232964>
- Moll, R. H., Kamprath, E. J., & Jackson, W. A. (1982). Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. *Agronomy Journal*, 74, 562–564. <https://doi.org/10.2134/agronj1982.00021962007400030037x>
- Moradtalab, N., Weinmann, M., Walker, F., Höglinger, B., Ludewig, U., & Neumann, G. (2018). Silicon improves chilling tolerance during early growth of maize by effects on micronutrient homeostasis and hormonal balances. *Frontiers in Plant Science*, 9, Article 420. <https://doi.org/10.3389/fpls.2018.00420>
- Nadeem, M., Mollier, A., Morel, C., Vives, A., Prud'homme, L., & Pellerin, S. (2011). Relative contribution of seed phosphorus reserves and exogenous phosphorus uptake to maize (*Zea mays* L.) nutrition during early growth stages. *Plant and Soil*, 346, 231–244. <https://doi.org/10.1007/s11104-011-0814-y>
- Nadeem, M., Mollier, A., Morel, C., Vives, A., Prud'homme, L., & Pellerin, S. (2012). Maize (*Zea mays* L.) endogenous seed phosphorus remobilization is not influenced by exogenous phosphorus availability during germination and early growth stages. *Plant and Soil*, 357(1-2), 13–24. <https://doi.org/10.1007/s11104-011-1111-5>
- Ozturk, L., Eker, S., Torun, B., & Cakmak, I. (2005). Variation in phosphorus efficiency among 73 bread and durum wheat genotypes grown in a phosphorus-deficient calcareous soil. *Plant and Soil*, 269, 69–80. <https://doi.org/10.1007/s11104-004-0469-z>
- Pang, J., Bansal, R., Zhao, H., Bohuon, E., Lambers, H., Ryan, M. H., & Siddique, K. H. M. (2018). The carboxylate-releasing phosphorus-mobilizing strategy can be proxied by foliar manganese concentration in a large set of chickpea germplasm under low phosphorus supply. *The New Phytologist*, 219, 518–529. <https://doi.org/10.1111/nph.15200>
- Parentoni, S. N., & de Souza Júnior, C. L. (2008). Phosphorus acquisition and internal utilization efficiency in tropical maize genotypes. *Pesquisa Agropecuária Brasileira*, 43, 893–901. <https://doi.org/10.1590/S0100-204X2008000700014>
- Pearse, S. J., Veneklaas, E. J., Cawthray, G., Bolland, M. D. A., & Lambers, H. (2007). Carboxylate composition of root exudates does not relate consistently to a crop species' ability to use phosphorus from aluminium, iron or calcium phosphate sources. *The New Phytologist*, 173, 181–190. <https://doi.org/10.1111/j.1469-8137.2006.01897.x>
- Piepho, H.-P. (2009). Data transformation in statistical analysis of field trials with changing treatment variance. *Agronomy Journal*, 101, 865–869. <https://doi.org/10.2134/agronj2008.0226x>
- Postma, J. A., Dathe, A., & Lynch, J. (2014). The optimal lateral root branching density for maize depends on nitrogen and phosphorus availability. *Plant Physiology*, 166, 590–602. <https://doi.org/10.1104/pp.113.233916>
- Postma, J. A., & Lynch, J. (2011). Root cortical aerenchyma enhances the growth of maize on soils with suboptimal availability of nitrogen, phosphorus, and potassium. *Plant Physiology*, 156, 1190–1201. <https://doi.org/10.1104/pp.111.175489>

- Raghothama, K. G., & Karthikeyan, A. S. (2005). Phosphate acquisition. *Plant and Soil*, 274, 37–49. <https://doi.org/10.1007/s11104-004-2005-6>
- Reinhart, K. O., Wilson, G. W. T., & Rinella, M. J. (2012). Predicting plant responses to mycorrhizae: Integrating evolutionary history and plant traits. *Ecology Letters*, 15, 689–695. <https://doi.org/10.1111/j.1461-0248.2012.01786.x>
- Richardson, A. E., & Simpson, R. J. (2011). Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiology*, 156, 989–996. <https://doi.org/10.1104/pp.111.175448>
- Rincint, R., Nicolas, S., Bouchet, S., Altmann, T., Brunel, D., Revilla, P., & Charcosset, A. (2014). Dent and Flint maize diversity panels reveal important genetic potential for increasing biomass production. *Theoretical and Applied Genetics*, 127, 2313–2331. <https://doi.org/10.1007/s00122-014-2379-7>
- Sattari, S. Z., Bouwman, A. F., Giller, K. E., & van Ittersum, M. K. (2012). Residual soil phosphorus as the missing piece in the global phosphorus crisis puzzle. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 6348–6353. <https://doi.org/10.1073/pnas.1113675109>
- Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W., & Zhang, F. (2011). Phosphorus dynamics: From soil to plant. *Plant Physiology*, 156, 997–1005. <https://doi.org/10.1104/pp.111.175232>
- Shenoy, V. V., & Kalagudi, G. M. (2005). Enhancing plant phosphorus use efficiency for sustainable cropping. *Biotechnology Advances*, 23, 501–513. <https://doi.org/10.1016/j.biotechadv.2005.01.004>
- Smith, S. E., Jakobsen, I., Grønlund, M., & Smith, F. A. (2011). Roles of arbuscular mycorrhizas in plant phosphorus nutrition: Interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiology*, 156, 1050–1057. <https://doi.org/10.1104/pp.111.174581>
- Tang, H., Chen, X., Gao, Y., Hong, L., & Chen, Y. (2020). Alteration in root morphological and physiological traits of two maize cultivars in response to phosphorus deficiency. *Rhizosphere*, 14, Article 100201. <https://doi.org/10.1016/J.RHISPH.2020.100201>
- Unterseer, S., Pophaly, S. D., Peis, R., Westermeier, P., Mayer, M., Seidel, M. A., & Schön, C.-C. (2016). A comprehensive study of the genomic differentiation between temperate Dent and Flint maize. *Genome Biology*, 17(1), Article 137. <https://doi.org/10.1186/s13059-016-1009-x>
- Van der Heijden, M. G. A., Martin, F. M., Selosse, M.-A., & Sanders, I. R. (2015). Mycorrhizal ecology and evolution: The past, the present, and the future. *The New Phytologist*, 205, 1406–1423. <https://doi.org/10.1111/nph.13288>
- Vance, C. P., Uhde-Stone, C., & Allan, D. L. (2003). Phosphorus acquisition and use: Critical adaptations by plants for securing a nonrenewable resource. *The New Phytologist*, 157, 423–447. <https://doi.org/10.1046/j.1469-8137.2003.00695.x>
- Wang, X., Shen, J., & Liao, H. (2010). Acquisition or utilization, which is more critical for enhancing phosphorus efficiency in modern crops? *Plant Science*, 179, 302–306. <https://doi.org/10.1016/j.plantsci.2010.06.007>
- Wang, Y.-L., Almvik, M., Clarke, N., Eich-Greatorex, S., Øgaard, A. F., Krogstad, T., & Clarke, J. L. (2015). Contrasting responses of root morphology and root-exuded organic acids to low phosphorus availability in three important food crops with divergent root traits. *AoB Plants*, 7, Article plv097. <https://doi.org/10.1093/aobpla/plv097>
- Wen, Z., Li, H., Shen, Q. I., Tang, X., Xiong, C., Li, H., Pang, J., Ryan, M. H., Lambers, H., & Shen, J. (2019). Tradeoffs among root morphology, exudation and mycorrhizal symbioses for phosphorus-acquisition strategies of 16 crop species. *The New Phytologist*, 223, 882–895. <https://doi.org/10.1111/nph.15833>
- White, P. J., & Veneklaas, E. J. (2012). Nature and nurture: The importance of seed phosphorus content. *Plant and Soil*, 357, 1–8. <https://doi.org/10.1007/s11104-012-1128-4>
- Wissuwa, M., Mazzola, M., & Picard, C. (2009). Novel approaches in plant breeding for rhizosphere-related traits. *Plant and Soil*, 321, 409–430. <https://doi.org/10.1007/s11104-008-9693-2>
- York, L. M., Galindo-Castañeda, T., Schussler, J. R., & Lynch, J. (2015). Evolution of US maize (*Zea mays* L.) root architectural and anatomical phenes over the past 100 years corresponds to increased tolerance of nitrogen stress. *Journal of Experimental Botany*, 66, 2347–2358. <https://doi.org/10.1093/jxb/erv074>
- Zemunik, G., Turner, B. L., Lambers, H., & Laliberté, E. (2015). Diversity of plant nutrient-acquisition strategies increases during long-term ecosystem development. *Nature Plants*, 1, Article 15050. <https://doi.org/10.1038/NPLANTS.2015.50>
- Zhang, J., Beusen, A. H. W., Van Apeldoorn, D. F., Mogollón, J. M., Yu, C., & Bouwman, A. F. (2017). Spatiotemporal dynamics of soil phosphorus and crop uptake in global cropland during the 20th century. *Biogeosciences*, 14, 2055–2068. <https://doi.org/10.5194/bg-14-2055-2017>
- Zhao, J. (2004). Characterization of root architecture in an applied core collection for phosphorus efficiency of soybean germplasm. *Chinese Science Bulletin*, 49, 1611–1620. <https://doi.org/10.1360/04wc0142>
- Zhu, J., & Lynch, J. (2004). The contribution of lateral rooting to phosphorus acquisition efficiency in maize (*Zea mays*) seedlings. *Functional Plant Biology*, 31, 949–958. <https://doi.org/10.1071/FP04046>
- Zhu, Y.-G., Smith, S. E., Barritt, A. R., & Smith, F. A. (2001). Phosphorus (P) efficiencies and mycorrhizal responsiveness of old and modern wheat cultivars. *Plant and Soil*, 237, 249–255. <https://doi.org/10.1023/A:1013343811110>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Li X, Mang M, Piepho H-P, Melchinger A, Ludewig U. Decline of seedling phosphorus use efficiency in the heterotic pool of flint maize breeding lines since the onset of hybrid breeding. *J Agro Crop Sci*. 2021;207:857–872. <https://doi.org/10.1111/jac.12514>