Received: 30 March 2022 | Revised: 20 August 2022 | Accepted: 23 August 2022
DOI: 10.1111/pbr.13054

ORIGINAL ARTICLE

Plant Breeding WILEY

How can we breed for phosphate efficiency in maize (*Zea mays*)?

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Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: 328017493/GRK 2366

Abstract

Future farming is required to produce high yields with reduced inputs. Increased fertilizer prices and policy goals underline the need to breed for nutrient-efficient varieties. We therefore conducted a multienvironmental field trial comprising 400 maize genotypes, half elite lines and half doubled haploid lines from six European landraces and assessed yield parameters and corresponding phosphorus concentrations at two developmental stages. From these traits, we derived several measures for phosphate efficiency and evaluated them phenotypically and genetically. The results of this study revealed that ample variation for phosphate efficiency is present in maize. However, while elite material clearly outperformed all landraces with regard to yield-related traits, some landrace genotypes indicated superior early development characteristics. The phosphate efficiency measures showed a complex genetic architecture, and hence, genomic selection appears best suited to assist their improvement. Taken together, breeding for phosphate efficiency is feasible but should be performed under the same conditions in which the crops are eventually grown because phosphate efficiency and what is deemed a sustainable P balance largely depends on the context.

KEYWORDS
breeding–genomic selection, landraces, maize, phosphorus–phosphate efficiency

1 | INTRODUCTION

Agriculture has to become more sustainable. While the need to improve agricultural production systems with regard to the social, economic and ecological framework is beyond controversy, the different ways how to reach this goal are still to be explored. In the European Union, the Farm to Fork strategy aims to considerably reduce chemical synthetic inputs for plant protection as well as fertilization (European Commission, 2020). In Germany, the concept of a ‘hybrid agriculture’, ensuring high yields while simultaneously delivering environmental services, is proposed as a future-oriented concept to achieve a resilient agricultural production (Future Committee Agriculture, 2021). Hence, plant breeders are in charge of developing high-yielding varieties that also perform under reduced inputs. Phosphorus (P) in the form of phosphate is the second most important plant macronutrient (Campbell & Reece, 2009). Due to the fact that Germany, for instance, has to import all of its applied phosphate fertilizer in a quantity of 253,478 t per year (FAOSTAT, 2019), the improvement of phosphate efficiency is a topical and crucial selection target in order to reach a sustainable P balance.

There are various definitions of the term phosphate efficiency including different calculations to measure it and one difficulty lies in their inconsistent deployment (Bovill et al., 2013; Rose & Wissuwa, 2012). In general, the trait phosphate use efficiency (PUE) can be split up into phosphate uptake efficiency (PUpE) and phosphate utilization efficiency (PUtE) (Leiser, Rattunde, Weltzien, & Haussmann, 2014; Parentoni & de Souza Júnior, 2008), a concept that was first proposed for nitrogen (Moll et al., 1982). Eventually, which phosphate efficiency measure is applied largely depends on the target environment. Hence, the breeding goals also have to adapt to different cultivation scenarios: While some regions of the world are challenged with very low soil P levels, for example, in sub-Saharan Africa, intensively farmed regions like Europe are instead confronted with eutrophication due to overfertilization (Withers et al., 2019). In the first case, low P concentrations in the harvested grain are desirable because soil P mining should be avoided (Leiser, Rattunde, Weltzien, & Haussmann, 2014) and P in the form of phytate negatively interacts with the uptake of other minerals and some micronutrients (Akhtar et al., 2018; Lux et al., 2022). In the case of high soil P levels, additional inputs in these sufficiently supplied agricultural soils should be reduced (Weiß et al., 2021). In both scenarios, high crop yields are aimed for, but the described scenarios also underline that no one-size-fits-all solution exists for P efficiency.

In this study, we cultivated a diverse set of maize genotypes under—compared with the common local farming practice—reduced P fertilizer inputs in order to better understand phosphate efficiency and which traits need to be tackled for a more sustainable agricultural production. We grew 400 maize genotypes in multienvironmental field trials in Germany and assessed them for their biomass yield at seedling stage and for biomass and grain yield at harvest and then analysed the P concentration in these tissues. Our objectives were to (1) calculate and evaluate different measures for phosphate efficiency, (2) investigate whether and in how far two elite heterotic groups and landraces differ with regard to phosphate efficiency, (3) evaluate the potential for genomics-assisted improvement of these traits and (4) draw conclusions for breeding programmes focussing on phosphate efficiency.

2 | MATERIAL AND METHODS

2.1 | Phenotypic trait assessment

2.1.1 | Germplasm

In this study, we used 400 maize (*Zea mays* L.) genotypes that have been described in detail previously (Weiß et al., 2022; Würschum et al., 2022). In brief, the investigated germplasm consisted of 200 elite lines (100 elite Flints [EF] and 100 elite Dents [ED]) and 200 European doubled haploid lines from landraces (11 individuals from Campan-Galade [CG], 33 from Gelber Badischer [GB], 14 from Sankt Galler Rheintaler [RT], 30 from Strenzfelder [SF], 53 from Satu Mare [SM] and 59 from Walliser [WA]). Due to heterogeneity of one genotype, which became obvious during the field trials, this genotype was excluded from the analyses and the final number of genotypes amounted to 399.

2.1.2 | Field trials

For a reliable trait assessment, multiple environmental field trials were performed in an alpha lattice design (VSN International, 2018) comprising two replicates per genotype. Each plot had a net size of 6 m^2, and plant density was controlled to 8.66 plants/m^2. In 2019, the trial was conducted at the experimental station Heidfeldhof of the University of Hohenheim (Lat 48.71117, Long 9.19594). In 2020, in addition to Heidfeldhof, the breeding station Eckartsweier served as another location (Lat 48.51974, Long 7.87076). Both locations are characterized in detail in Table S1. It should be mentioned that all fields where the experiments were conducted, showed typical base P levels for the region, which are classified as optimal to high. Furthermore, the weather data (Agrometeorology Baden-Württemberg, 2022) for both stations and both years are depicted in Figure S1. It can be seen that the spring of 2019 was extraordinary cold (May 2019: -2.6°C compared with the long-term mean) and wet (May 2019: $+89.5\%$) in Hohenheim. This represented challenging conditions for maize, especially with regard to acquiring sufficient P during the early developmental phase, when seed P resources have already been used up.

The traits relevant for phosphate efficiency measures were assessed in three different sample materials: seedling (abbreviated 'Se', four plants per genotype were sampled at growth stages V4–V6), stover (abbreviated 'St', three plants per genotype were sampled and manually separated from cobs at harvest) and grain (all plants of the 6 m^2 plot were threshed at physiological maturity with a combine harvester). It should be mentioned that a separate harvest of grain and stover did not take place in the field season 2019; therefore, all measures based on stover refer to the two trials in 2020.

From the field, we generally obtained seedling (SeY in g/4 plants), stover (StY in g/plant) and grain yield (GY in t/ha) parameters. The harvested samples were dried and thus represent dry matter biomass of seedling and stover, while in the case of grain yield, the plot yield was corrected for dry matter content. In the lab, all samples were analysed for P concentration (Pconc in ppm) by X-ray fluorescence (Bruker, Billerica, MA, USA). Based on these field and lab data, all other measurements were calculated on a plot basis (for raw data see Table S2).

2.2 | Data analysis

If not stated otherwise, RStudio version 3.5.3 was used for the statistical analyses (RStudio Team, 2020).

2.2.1 | Estimation of parameters

As described in detail in a previous study (Weiß et al., 2022), the first step was to remove outliers from the dataset. Then, a mixed model was applied for the three environments jointly to derive variance components, heritabilities and best linear unbiased estimates (BLUEs) using the software tool ASReml, version 3.0 (Butler et al., 2009). For the calculation of the subgroup-specific variance parameters, we introduced dummy variables that assigned each genotype to the corresponding subgroup. Broad-sense heritability was calculated following Hallauer et al. (2010). Analyses of variance for each trait were calculated by the R package 'agricolae' (de Mendiburu, 2020).

2.2.2 | Genome-wide association mapping and genomic prediction

The 50K marker data of all genotypes (available at Weiß et al., 2022) were first subjected to a quality control with regard to missing values (<20%), heterozygotes (<5%) and monomorphic markers. Imputation using Beagle 5.2 (Browning et al., 2018) was done separately for the three groups ED, EF and landraces. After imputation files were merged and afterwards filtered for a minor allele frequency >5%. This resulted in a total of 22,101 SNP markers and consequently a Bonferroni-corrected significance threshold of 5.65 $[-\log_{10}(0.05/22,101)]$. The so obtained set of markers was used to perform genome-wide association mapping for each of the investigated traits using the Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK) approach (Huang et al., 2019) available in 'GAPIT 3.0' (Wang & Zhang, 2021). The physical position of markers referred to the B73 reference genome, version 4 (Jiao et al., 2017).

We also applied genomic prediction by rrBLUP (Endelman, 2011). In order not to intermingle differences in population mean performance with prediction accuracy, we performed this analysis in the four largest groups, that is, the ED, the EF and the two largest landraces SM and WA separately. We used fivefold cross-validation with 1000 runs to calculate the Pearson correlation coefficients between estimated and observed trait values and divided this predictive ability by the square root of the heritability to obtain the prediction accuracy (Zhao et al., 2012).

The presentation of data was facilitated through the packages 'ggpubr' (Kassambara, 2020), 'devtools' (Wickham et al., 2021), 'gghalves' (Tiedemann, 2020), 'ggbiplot' (Vu, 2011), 'qqman' (Turner, 2018), 'qgraph' (Epskamp et al., 2012) and 'tidyverse' (Wickham et al., 2019). All graphical codes were run with RStudio version 4.1.0.

3 | RESULTS

3.1 | How to capture phosphate efficiency?

Screening the literature for measures of phosphate efficiency revealed a plethora of different terms and formulae (Table 1). The different definitions can be grouped into the following categories: PUE, which itself can be separated into PUpE and PUtE. As a basis, all of them rely on a precise assessment of the field parameters, namely, yield measures as well as P concentration in the corresponding tissues. Additionally, P balance can be derived from these traits, which indicates how much P is in total removed from the field during harvest. Due to the fact that we did not supply any extra P fertilizers in our field trials, P balance corresponds to the negative value of the P content of the total biomass. After calculation of all listed phosphate efficiency measures, we excluded the ones that were highly (>0.99) correlated with each other for the sake of clarity. For the remaining 20 measures, we then analysed the summary statistics, variance components and heritabilities across environments (Table 2). When comparing the means, we found that the grain showed a significantly ($p < .01$) higher P concentration compared with the stover. The ratio of the genotypic (σ_g^2) to the genotype-by-environment interaction ($\sigma_{g\times e}^2$) variance components demonstrated for all measures the relative importance of the genotypic component compared with the genotype-by-environment interaction variance. Seedling yield showed the most substantial genotype-by-environment interaction, whereas P utilization of the biomass, which means how much aboveground biomass is produced per unit P, was largely determined by the genotype. Heritabilities were generally high, ranging from .63 for P content in the seedling to .92 for P utilization of the biomass, which confirmed an overall high quality of the phenotypic data. Furthermore, we also reported the genetic coefficient of variation, which puts the observed genotypic variance in relation to the mean of the corresponding trait. Here, we found the lowest coefficients for the traits P concentration in the grain, P-use efficiency of the grain and P quotient of utilization, whereas the highest genetic coefficient of variation was observed for grain yield. Collectively, phosphate efficiency can be described by several measures, which all showed ample genetic variation and high heritabilities.

3.2 | Evaluation of phosphate efficiency in diverse maize groups

We next investigated the relationships among the different phosphate efficiency measures (Figure 1). The biplot and the network plot (Figure 1a,b) revealed that the applied measures cover different aspects of phosphate efficiency and are more or less strongly related to each other. These correlations were in part dependent on the tissue type, as for example seedling and stover measures formed somewhat distinct groups. Interestingly, the landrace SM seems to contain a wide diversity, including specifically high performers for the P utilization of stover (Figure 1a). Also, landraces showed generally higher trait values for the P concentrations in grain and stover.

As plant breeding usually focuses primarily on yield-related traits, the three yield measures seedling yield, stover yield and grain yield were investigated for their correlations with the different phosphate efficiency measures (Figure 1c). It can be noted that the correlation pattern of seedling yield differs considerably from that of the stover and grain yield, which themselves were positively and significantly correlated ($r = .46^{***}$) with each other. Higher P concentrations of the

TABLE 1 Different phosphate efficiency measures reviewed in literature

Category	Name	Abbr.	Calculation	Crop	Reference
PUE	P-use efficiency	**SeY** **StY** **GY**	$PUE = Yield$ $SeY = SeedlingYield$ $StY = StoverYield$ $GY = GrainYield$	Maize; rice	Adem et al. (2020); Azevedo et al. (2015)
PUE	Agronomic efficiency; P-use efficiency; partial factor productivity of P	agroPUE	$agroPUE = PUpE * PUtE = \frac{Yield}{P_{supplied}}$	Maize; sorghum	Frank Stephano et al. (2021); Leiser et al. (2015); Moll et al. (1982); Yan et al. (2021); Magalhaes (personal communication)
PUE	P-use efficiency	**PUE**	$PUE = \frac{1}{Pconc}$	Rice	Wissuwa (personal communication)
PUpE	P accumulation; P acquisition; P content; P uptake; whole-plant nutrient uptake	**Pcont G**	$PcontG = GY * Pconc_{Grain}$	Maize; rice; sorghum	Azevedo et al. (2015); Ciampitti and Vyn (2014); Leiser et al. (2015); Li, Chen, et al. (2021); Rose et al. (2016); Wissuwa and Ae (2001)
PUpE	P content; per-plant nutrient uptake	**Pcont Se** **Pcont St**	$PcontSe = \frac{SeY * Pconc}{Plant\ density}$ $PcontSt = \frac{StY * Pconc}{Plant\ density}$	Maize	Ciampitti and Vyn (2014)
PUtE	Physiological efficiency; P utilization efficiency; P utilization grain	**PUtE G**	$PUtE\ G = \frac{GY}{Pcont_{Biomass}}$	Maize; sorghum	Ciampitti and Vyn (2014); Leiser et al. (2015); Moll et al. (1982); Parentoni and de Souza Júnior (2008)
PUtE	P utilization stover	**PUtE St**	$PUtE\ St = \frac{StY}{Pcont_{Biomass}}$	Sorghum; rice	Adem et al. (2020); Leiser et al. (2015)
PUtE	P utilization biomass	**PutE BM**	$PUtE\ BM = \frac{GY + StY}{Pcont_{Biomass}}$	Tomato; bean; sorghum	Gabelman and Gerloff (1983); Leiser et al. (2015)
PUtE	P ratio	**P ratio**	$P\ ratio = \frac{Pconc_{Grain}}{Pconc_{Stover}}$	Cereals	Zhang et al. (2021)
PUtE	P harvest index	PHI	$PHI = \frac{Pcont_{Grain}}{Pcont_{Biomass}}$	Maize; sorghum	Leiser et al. (2015); Parentoni and de Souza Júnior (2008)
PUtE	P quotient of utilization	**QUTIL**	$QUTIL = \frac{GY}{Pcont_{Grain}}$	Maize	Parentoni and de Souza Júnior (2008)
PUtE	P internal utilization efficiency	PUTIL	$PUTIL = QUTIL * PHI$	Maize	Parentoni and de Souza Júnior (2008)
PUtE	P internal utilization efficiency	**PUtE int**	$PUtE\ int = \frac{BMY}{Pcont_{Stover}}$	Bean; rice	Ciampitti et al. (2013); Liao and Yan (1999); Rose et al. (2016)
Pbal	P balance	**Pbal**	$Pbal = P_{supplied} - P_{plant\ uptake}$	Maize	Redel et al. (2021)

Note: The categories phosphate use efficiency (PUE), phosphate uptake efficiency (PUpE), phosphate utilization efficiency (PUtE) and phosphate balance (Pbal) are distinguished. Yield stands, depending on the tissue, for seedling yield (SeY), stover yield (StY), grain yield (GY) or biomass yield (BMY, given as the sum of grain and stover). Accordingly, P concentration (Pconc) and P content (Pcont) can be specified according to the corresponding sample (seedling, grain, stover and biomass). Abbreviations, which were eventually used in this study, are highlighted in bold. PUE was calculated for all three tissues and together with the P concentrations for all tissues we end up with 20 measures in total.

seedling were positively correlated with seedling, stover and grain yield. Additionally, a high seedling yield, and thus a good early development, was associated with higher P concentrations not only in the seedling, but also in stover and grain. All yield parameters showed a negative correlation with PUE in the seedling as well as with P balance. The calculation of P content is based on the P concentration and yield of the respective tissue, which themselves showed a positive correlation of $.18^{***}$ for the seedling and negative correlations of $-.27^{***}$ for stover and $-.50^{***}$ for grain. We found that P content was highly positively correlated with yield for all tissue types ($.69^{***} < r < .99^{***}$), but P content of the grain showed a negative correlation of $-.39$ with P concentration in the grain.

In order to capture phosphate efficiency, we exemplarily chose six traits from different clusters of the network plot for more detailed analyses: seedling yield, P concentration of the seedling, grain yield, P-use efficiency of the stover, P quotient of utilization and P balance. Jointly, these traits can be used to describe the complex trait phosphate efficiency and cover it from different angles. Because our panel is composed of different breeding material, it also allows to evaluate the performance of each subgroup (Figure 2 and Table S3). For the six exemplary traits, the most prominent and significant differences can be seen for grain yield and P balance, where the elite lines are clearly distinct from the landraces, as they showed generally higher grain yields and therefore a more negative P balance. Interestingly, the

TABLE 2 Summary statistics for all phosphate efficiency measures

Variable	Unit	Mean	σ_g^2	$\sigma_{g\times e}^2$	σ_e^2	$\sigma_g^2:\sigma_{g\times e}^2$	H^2	GCV
SeY♣	(g/4 plants)	6.42	1.91	1.47	1.43	1.30	.72	.21
Pconc seedling♣	(ppm)	3176.38	96,799.02	63,288.92	117,411.66	1.53	.70	.10
StY	(g/plant)	41.96	183.31	16.69	82.83	10.98	.86	.32
Pconc Stover	(ppm)	2161.83	265,117.83	30,374.25	88,047.95	8.73	.88	.24
GY♣	(t/ha)	1.84	1.35	0.58	0.16	2.31	.86	.63
Pconc Grain♣	(ppm)	3181.55	52,688.89	11,733.83	23,984.76	4.49	.87	.07
PUE Se♣	(kg/g P)	0.34	1.30E-03	8.94E-04	1.59E-03	1.46	.70	.11
PUE St	(kg/g P)	0.53	1.88E-02	6.75E-03	6.78E-03	2.78	.79	.26
PUE G♣	(kg/g P)	0.32	5.48E-04	1.37E-04	2.34E-04	4.00	.87	.07
Pcont Se♣	(g/plant)	0.01	1.32E-06	1.58E-06	1.50E-06	0.84	.63	.21
Pcont St	(g/plant)	0.09	6.83E-04	2.52E-04	5.54E-04	2.71	.72	.30
Pcont G♣	(kg/ha)	5.58	11.43	4.23	1.77	2.70	.87	.61
Pcont BM	(kg/ha)	12.41	15.20	2.39	5.04	6.35	.86	.31
PUtE St	(g/mg P)	0.32	8.60E-03	9.76E-04	2.12E-03	8.81	.89	.29
PUtE G	(kg/g P)	0.11	2.93E-03	6.96E-04	6.97E-04	4.21	.85	.50
PUtE BM	(kg/g P)	0.42	6.97E-03	4.03E-04	1.50E-03	17.28	.92	.20
P ratio	(%)	172.17	1580.12	486.12	797.76	3.25	.78	.23
QUTIL♣		318.42	557.6	145.44	230.17	3.83	.87	.07
PUtE int		776.50	78,312.58	43,588.6	32,097.38	1.80	.72	.36
Pbal	(kg/ha)	−12.41	15.20	2.39	5.04	6.35	.86	−.31

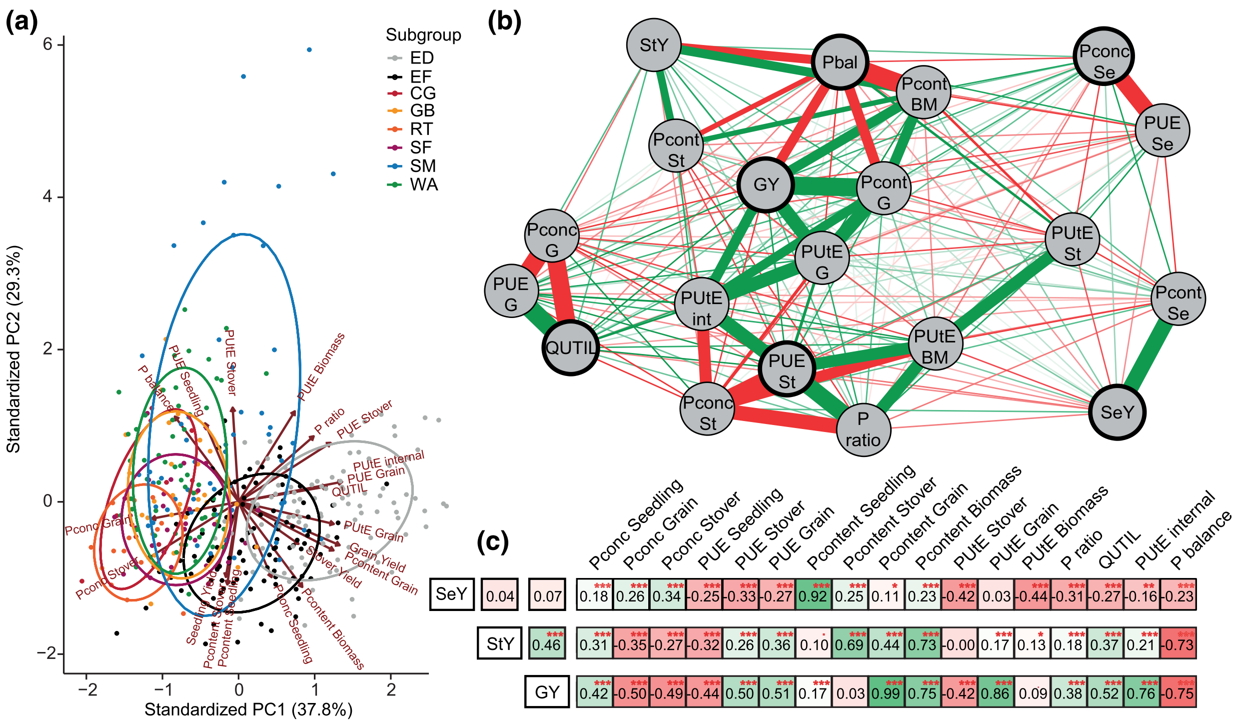
Note: σ_g^2 denotes the genotypic variance, $\sigma_{g\times e}^2$ the genotype-by-environment interaction variance, σ_e^2 the error variance and H^2 the broad-sense heritability. The genetic coefficient of variation is abbreviated as GCV. Traits are named as follows: seedling yield (SeY); P concentration in seedling (Pconc Seedling); stover yield (StY); P concentration in stover (Pconc Stover); grain yield (GY); P concentration in grain (Pconc Grain); phosphate use efficiency of seedling (PUE Se), of stover (PUE St) and of grain (PUE G); P content seedling (Pcont Se), stover (Pcont St), grain (Pcont G) and biomass (Pcont BM); phosphate utilization efficiency of stover (PUtE St), of grain (PUtE G), of biomass (PUtE BM), P ratio, P quotient of utilization (QUTIL), P internal utilization efficiency (PUtE int) and P balance (Pbal). Traits based on all three location–year–combinations (Hohenheim 2019 and 2020, Eckartsweier 2020) are marked with a ♣. Otherwise, they are based on Hohenheim 2020 and Eckartsweier 2020.

landraces RT, GB and SF showed a superior early development. More generally speaking, early plant development, represented by seedling yield, clearly separated the lower performing Dent from the Flint pool (EF and all landraces). Moreover, the two subgroups ED and SM behaved differently than the other groups. While both demonstrated a below-average performance in the early stages, they had a significantly higher PUE of the stover compared with the other subgroups. The ED material also showed the highest quotient of P utilization. Moreover, if we select of all 20 indicators for phosphate efficiency the 10% genotypes with the highest trait values of the whole panel and subsequently filter out the 10% genotypes with the highest occurrence, we end up exclusively with ED lines except for two EF and one SM genotype. Taken together, the germplasm and the analysed tissue have a major impact on the outcome of phosphate efficiency indicators.

3.3 | The genetic architecture of phosphate efficiency measures

As a first step, we performed genome-wide association mapping to identify quantitative trait loci (QTL). Across all traits, this revealed only few single nucleotide polymorphism (SNP) markers that surpassed the Bonferroni-corrected significance threshold of 5.65. For the two traits seedling yield and P utilization of the grain, no marker reached the significance threshold (Figure 3 and Table S4).

Due to the apparent quantitative nature of the traits, we next performed genomic prediction for all 20 traits in the four largest subgroups, namely, ED, EF, SM and WA (Figure 4). This showed that the three yield parameters generally achieved high prediction accuracies. One exception was grain yield in the two landraces, which was rather poorly predicted with .22 and .19. The trait P content of the biomass also resulted in high prediction accuracies for all four subgroups. Overall, the pattern for the two elite subgroups and for the two landraces appears quite similar. The highest prediction accuracy was obtained for the landrace WA with .73 for the P concentration in the seedling, while the lowest mean prediction accuracy of .17 was observed for SM for the P utilization of grain. Furthermore, when comparing the prediction accuracies of each subgroup, the ED showed the highest values across all traits with an average prediction accuracy of .53 and the landrace WA the lowest with an average prediction accuracy of .41. In summary, genomic prediction resulted for most phosphate efficiency measures in moderate to high prediction accuracies.



			Pconc Seedling	Pconc Grain	Pconc Stover	PUE Seedling	PUE Stover	PUE Grain	Pcontent Seedling	Pcontent Stover	Pcontent Grain	Pcontent Biomass	PUtE Stover	PUtE Grain	PUtE Biomass	P ratio	QUTIL	PUtE internal	P balance
SeY	0.04	0.07	0.18***	0.26***	0.34***	-0.25***	-0.33***	-0.27***	0.92***	0.25***	0.11*	0.23***	-0.42***	0.03	-0.44***	-0.31***	-0.27***	-0.16**	-0.23***
	StY	0.46***	0.31***	-0.35***	-0.27***	-0.32***	0.26***	0.36***	0.10.	0.69***	0.44***	0.73***	-0.00	0.17***	0.13*	0.18***	0.37***	0.21***	-0.73***
		GY	0.42***	-0.50***	-0.49***	-0.44***	0.50***	0.51***	0.17***	0.03	0.99***	0.75***	-0.42***	0.86***	0.09	0.38***	0.52***	0.76***	-0.75***

FIGURE 1 Relationships among phosphate efficiency indicators. (a) Biplot of all 20 traits and measures with the 399 genotypes assigned to their subgroups, that is, elite Dents (ED), elite Flints (EF), Campan-Galade (CG), Gelber Badischer (GB), St. Galler Rheintaler (RT), Strenzfelder (SF), Satu Mare (SM) and Walliser (WA). (b) Network correlation plot. The line between two traits depicts the strength of the Pearson correlation coefficient, red showing negative, green positive correlations. Traits are abbreviated as seedling yield (SeY); P concentration in seedling (Pconc Se); stover yield (StY); P concentration in stover (Pconc St); grain yield (GY); P concentration in grain (Pconc G); phosphate use efficiency seedling (PUE Se), stover (PUE St) and grain (PUE G); P content of seedling (Pcont Se), of stover (Pcont St), of grain (Pcont G) and of biomass (Pcont BM); phosphate utilization efficiency of stover (PUtE St), grain (PUtE G) and biomass (PUtE BM); P ratio; P quotient of utilization (QUTIL); P internal utilization efficiency (PUtE int); and P balance (Pbal). Six exemplary traits from different clusters are circled in bold and used for further analyses. (c) Pearson correlation matrix for the yield measures with the other phosphate efficiency measures. Significance levels are shown as '*' (*p* value <0.05), '**' (*p* value < .01), '***' (*p* value <.001). [Color figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

4.1 | Phosphate efficiency is context dependent

As already mentioned in the introduction, the definition and prioritization of phosphate efficiency measures are strongly dependent on the available P in the target environment and on the harvested material being removed from the field. While the first factor is mainly influenced by the base P level and the soil type with its properties (Erel et al., 2017; Ibrahim et al., 2021; Leiser, Rattunde, Weltzien, Cisse, et al., 2014), the amount of fertilizer and the interaction of P with other plant nutrients (Frank Stephano et al., 2021; Weiß et al., 2021) as well as the management of the field (Nkebiwe et al., 2016; Xin et al., 2017), the latter is above all a question of the farm's operating mode. A dairy farm will harvest silage maize and in the next season resupply a part of the P to its fields in form of manure. By contrast, an agricultural farm that sells maize grain for human or animal consumption exports the therein contained nutrients and therefore removes them from the farm's P cycle. Hence, it is especially desirable and relevant for pure crop farms to realize adequate P levels of their fields. This example emphasizes the point how strongly the definition of phosphate efficiency is dependent on the context, even within a given environment. Generally, PUpE is deemed more important in P deficient environments, whereas PUtE is considered more relevant in P rich environments (Bovill et al., 2013).

In Europe, most fields were fertilized sufficiently with P in the course of the last decades and the soil P status is high (Table S1), also in a global comparison. Interestingly, high P concentrations in the seedling seem to result in a higher seedling, stover and grain yield, which is likely due to an improved seedling vigour. This underlines the importance of early development in maize as the uptake of P is most critical in this stage. Moreover, stover and grain yield in maize were mostly positively correlated with the investigated phosphate efficiency measures. Unlike other negatively correlated target traits in crops that impose a trade-off during selection, as for example protein content and grain yield (Neuweiler et al., 2021), breeders can benefit from the fact that the selection for grain yield indirectly has resulted in a concomitant improvement of most of the PUE measures. Thus, breeding for phosphate efficiency in a European context, which means realizing high yields under the given soil P levels without extra P fertilizer inputs, appears feasible with the available genetic variation and does not seem to come at the expense of other target traits.

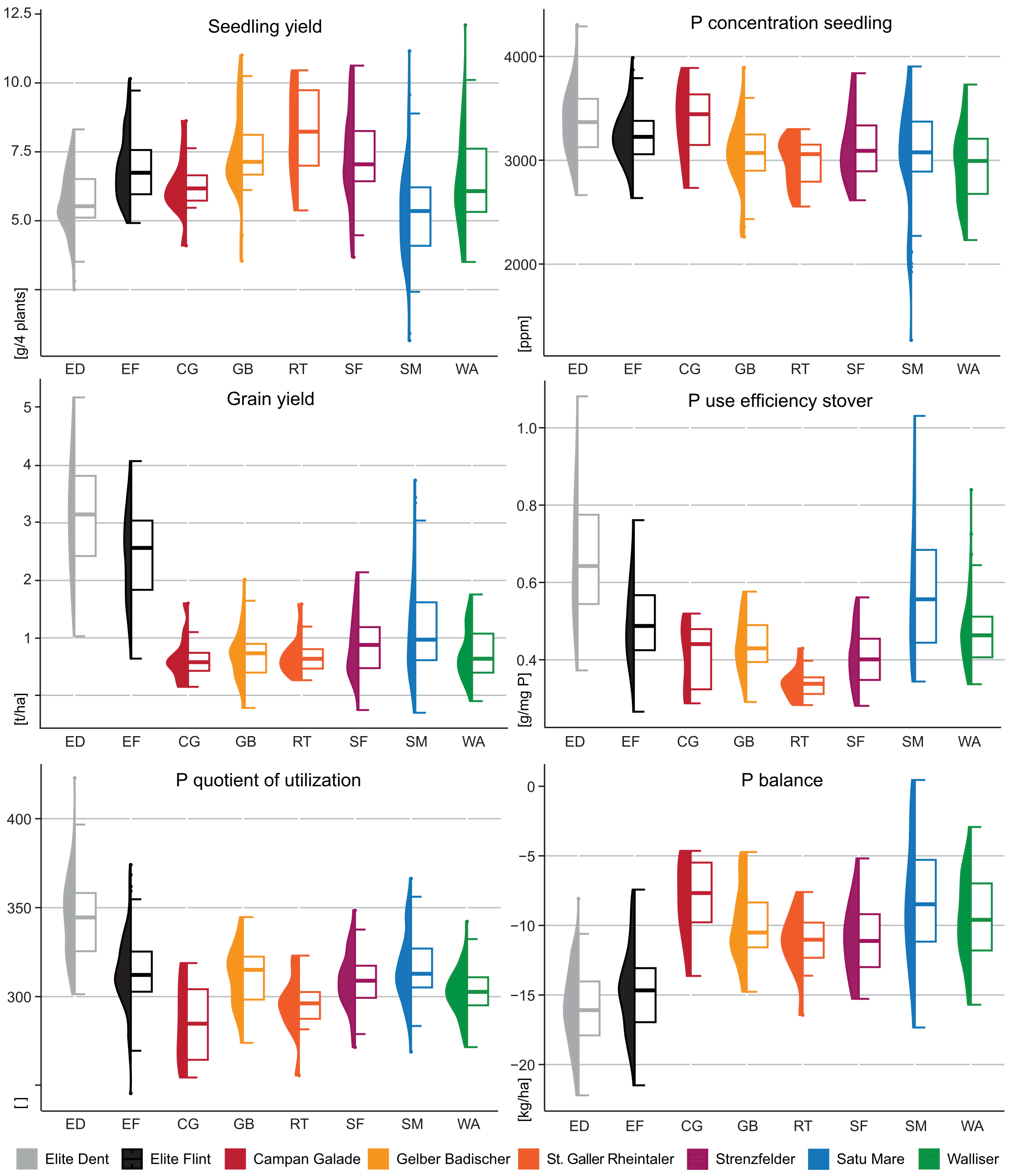


FIGURE 2 Phenotypic distribution of six exemplary traits representing the phosphate efficiency measures, shown for the different subgroups [Color figure can be viewed at wileyonlinelibrary.com]

4.2 | Impact of the breeding material on phosphate efficiency

The differences between the subgroups became evident in the biplot and the phenotypic distribution of the investigated traits (Figures 1a and 2 and Table S3). We observed a generally superior performance of ED lines in comparison with the Flint subgroups, including a substantially higher grain yield. Except for a significantly lower seedling yield of the ED lines due to their lower cold tolerance (Strigens et al., 2013), Dent genotypes therefore also showed generally higher trait values with regard to phosphate efficiency indicators. Previous studies have shown that American Dents display a higher polymorphic richness than European Flint lines (Rebourg et al., 2003; Revilla et al., 2014). This fact and the overall longer breeding history of Dent lines may explain the higher genetic gains achieved in Dent material compared with Flints and therefore a higher line per se performance under favourable conditions (Moreno-Gonzalez et al., 1997). Moreover, the SM landrace lines showed extraordinarily high trait values for PUtE of the stover. The reason for this is that SM genotypes frequently showed the phenomenon of barren stalks. Because no cobs were formed, then the ratio of stover yield to total P in the biomass was distorted, which led to such artificially high values for PUtE of the stover.

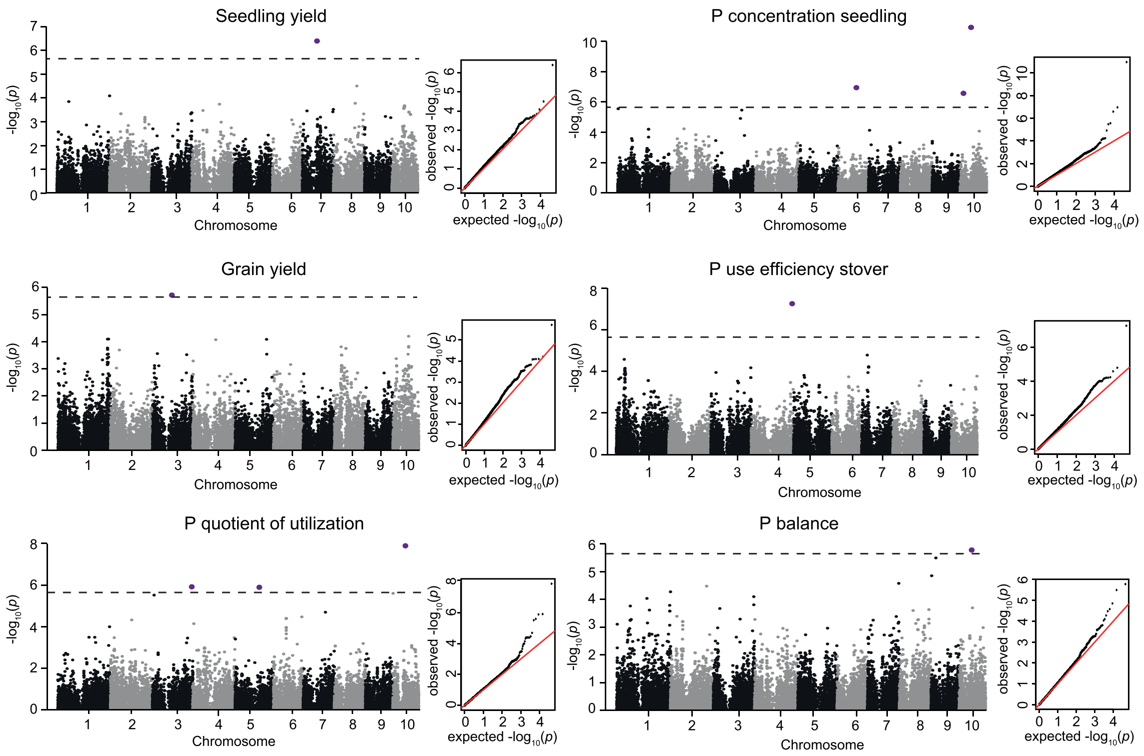


FIGURE 3 Results from genome-wide association mapping performed with 22,101 markers for the six exemplary traits. Manhattan plots and QQ plots are shown, the Bonferroni-corrected significance threshold is indicated as horizontal dashed line, and significant SNPs are highlighted in purple. [Color figure can be viewed at wileyonlinelibrary.com]

It should be noted that the choice of the investigated measures we use as selection criteria will have a decisive impact on the composition of the selected fraction. Performing a combined selection across all traits resulted in almost only Dent lines. Because many phosphate efficiency measures contain the factor yield, elite material is clearly superior and will always be preferred. While heterotic pools, such as Flint and Dent in our example, will be kept separate anyhow during breeding, this nevertheless illustrates that the genetic background should be kept in mind when working with different material groups as the landraces in our study.

4.3 | The potential of marker-assisted breeding for phosphate efficiency

According to Gojon et al. (2022), only few major QTL for phosphate efficiency were reported in maize (Chen et al., 2009; Li, Wang, et al., 2021). Notably, studies where major QTL could be detected were performed under severely P-deficient conditions. The identification of the underlying causal genes for phosphate efficiency in crops was so far only achieved for very few cases. *PSTOL1* in rice (*Oryza sativa* L.) represents the probably most well-known example. This gene acts as an enhancer of early root growth and root surface area and therefore improves phosphate uptake under low P conditions (Gamuyao et al., 2012; Wissuwa et al., 1998). Another example is *SbMATE* in sorghum (*Sorghum bicolor* L. Moench), known to be causal for aluminium tolerance, that was proposed to have a pleiotropic effect also on grain yield under P deficiency (Leiser, Rattunde, Weltzien, Cisse, et al., 2014). Our study, which took place under sufficient P supply, did not identify any consistent QTL for the investigated phosphate efficiency indicators. This is somehow to be expected as most of the traits contain or are derived from yield, which is a trait with a highly quantitative nature. In line with this, we observed moderate to high accuracies for genomic prediction, which varied somewhat depending on the genetic group and the chosen phosphate efficiency indicator. Nevertheless, given appropriate training set sizes and designs, our results illustrate the potential of genomic selection to assist the improvement of phosphate efficiency in breeding.

4.4 | Breeding for a sustainable P balance

Because breeding means thinking ahead, also breeders in countries with currently well P-supplied soils should already now work on varieties that will perform well under reduced P availability, as reduced P fertilizer inputs will eventually lead to lower P base levels in the soil. Taking into consideration how much phosphate efficiency depends on the context, we recommend to conduct the selection of phosphate-efficient crops under the target conditions under which the crops will eventually be grown. Breeding under reduced P field conditions seems reasonable in order to select genotypes that possess characteristics to perform well under such conditions. For example, a recent study has

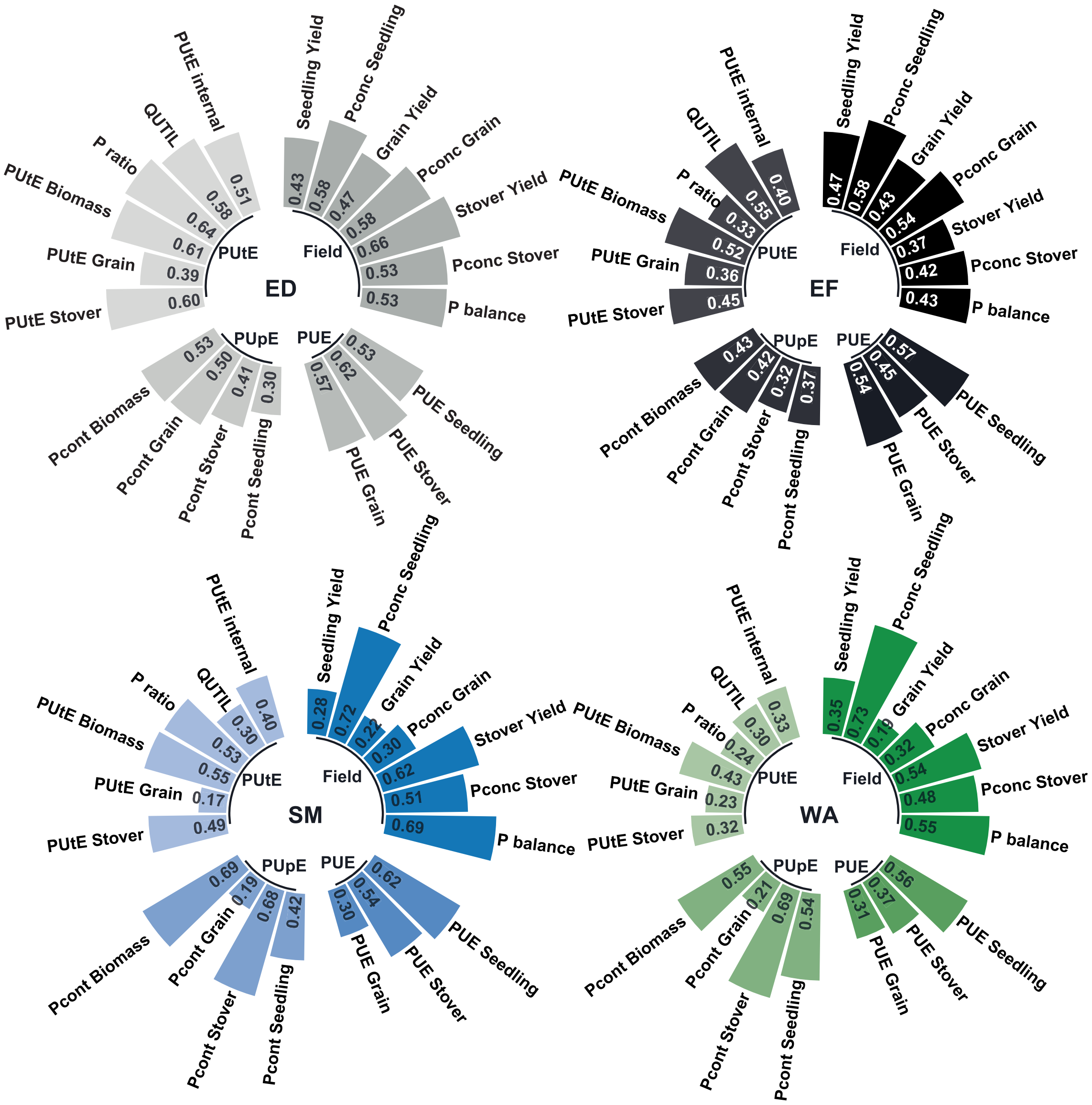


FIGURE 4 Genomic prediction results for the 20 phosphate use efficiency components and measures, split up into the category 'Field', phosphate use efficiency (PUE), phosphate uptake efficiency (PUpE) and phosphate utilization efficiency (PUtE). Results are given as prediction accuracy obtained with 1000 cross-validation runs. Subgroups are abbreviated as elite Dents (ED), elite Flints (EF) and the two landraces Satu Mare (SM) and Walliser (WA). [Color figure can be viewed at wileyonlinelibrary.com]

demonstrated that cultivating maize without extra starter fertilizers in breeding nurseries can be recommended as there is sufficient genetic variation to tolerate this reduced fertilizer input (Weiß et al., 2021). Moreover, future research concerning phosphate, or generally speaking nutrient efficiency, should take root traits into account, which may also be promising with regard to the increased need for drought tolerant maize (Hund et al., 2009).

Finally, in breeding programmes that usually deal with large numbers of genotypes, we need traits that can be easily scored and are thus amenable to high throughput. Therefore, the crucial question with regard to screening phosphate efficiency is: Which trait to choose? Of the 20 phosphate efficiency indicators investigated in this study, we observed fairly high heritabilities and genomic predictive accuracies for the field-related traits (Figure 4). For this reason, we recommend to focus on the traits seedling, stover and grain yield as well as the P concentration in all three tissues as a straightforward approach to assess phosphate efficiency. Specific selection strategies and breeding objectives can then be adapted to the respective conditions. While yield at harvest is already routinely assessed, analysing P concentration at the seedling stage would also enable an indirect selection for the improvement of all yield measures (Figure 1c). However, the tissue sampling and measuring the P concentration may not be applicable to the many selection candidates in early generations. Thus, genomic selection appears promising to target P concentration in early generations in a resource- and time-efficient manner (Weiß et al., 2022).

In addition, it should be noted that it also matters in which form P is present in the plants (Rodehutscord et al., 2016). For food and feed alike, a reduced concentration of phytate or an increased phytase activity is desirable to ensure a sufficient mineral supply (Akhtar et al., 2018; Humer & Zebeli, 2015). In conclusion, this study illustrated that it is necessary to define what exactly is meant by sustainable P balance because this may vary greatly depending on the context. Importantly, however, breeding for phosphate efficiency is feasible as illustrated by the available genetic variation for the different measures, which allows the targeted selection depending on the specific environmental requirements. Interestingly, Flint landraces showed valuable characteristics with regard to early development, which illustrates that for some traits the introgression of such beneficial variation from more exotic material into elite breeding material may be worthwhile. As phosphate efficiency generally showed a complex genetic architecture, a combination of genomic and phenotypic selection appears most suitable for the improvement of phosphate efficiency in breeding towards a more sustainable agriculture.

ACKNOWLEDGEMENTS

The authors thank all technical staff members for their excellent work to generate the field and laboratory data, namely, from the Institute of Plant breeding Mire Halilaj, Jochen Jesse, Franz Josef Mauch, Sebastian Pluskat, Heralt Pöschel and Regina Volkhausen as well as from the State Plant Breeding Institute Regina Bauer and Christiane Maus. The German Research Foundation (Deutsche Forschungsgemeinschaft [DFG]) financed this project, research grant 328017493/GRK 2366 (International Research Training Group AMAIZE-P 'Adaptation of maize-based food-feed-energy systems to limited phosphate resources'). Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Tobias Würschum and Willmar L. Leiser. *Methodology*: Tobias Würschum and Willmar L. Leiser. *Formal analysis*: Thea M. Weiß and Dongdong Li. *Investigation*: Thea M. Weiß. *Data curation*, Thea M. Weiß. *Writing—Original draft preparation*: Thea M. Weiß and Tobias Würschum. Writing—review and editing: Thea M. Weiß, Willmar L. Leiser, Dongdong Li, Sandra Roller, Wenxin Liu, Volker Hahn and Tobias Würschum. *Visualization*: Thea M. Weiß and Tobias Würschum. *Supervision*: Tobias Würschum, Willmar L. Leiser and Wenxin Liu. *Funding acquisition*: Tobias Würschum.

DATA AVAILABILITY STATEMENT

Data are openly available in a public repository that issues datasets with DOIs.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Weiß, T. M., Li, D., Roller, S., Liu, W., Hahn, V., Leiser, W. L., & Würschum, T. (2022). How can we breed for phosphate efficiency in maize (*Zea mays*)? *Plant Breeding*, *141*(6), 733–744. https://doi.org/10.1111/pbr.13054