UNIVERSITY OF HOHENHEIM

Assessing the genetic variation of phosphate efficiency in European maize (Zea mays L.)

Dissertation to obtain the doctoral degree of Agricultural Sciences

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Meiner lieben Mutter,

die mich für die Landwirtschaft begeistert hat

Contents

Abbreviations

CAN	Calcium ammonium nitrate
DAP	Diammonium phosphate
DAS	Days after sowing
DH	Doubled haploid
GDM	Grain dry matter content
GS	Genomic prediction *or* selection
GWAS	Genome-wide association study
GY	Grain yield
IP6	Phytate
MAF	Minor allele frequency
N	Nitrogen
NIRS	Near-infrared spectroscopy
P	Phosphorus *or* phosphate
PHT	Phosphate transporter
Pi	Inorganic phosphorus
Po	Organic phosphorus
PS	Phenomic prediction *or* selection
PUpE	Phosphorus uptake efficiency
PUtE	Phosphorus utilization efficiency
QTL	Quantitative trait locus
SNP	Single nucleotide polymorphism
TSP	Triple superphosphate
XRF	X-ray fluorescence

“Beim Betrachten eines Düngerhaufens
Er ist der Schatz und das Kleinod des Bauern, die breiteste Grundlage für die Landwirtschaft und somit ein Faktor der Staatserhaltung […] und der bestehenden Ordnung.“

"When looking at a pile of fertilizer
It is the treasure and jewel of the farmer, the broadest basis for agriculture and thus a factor in the preservation of the state [...] and the existing order".

(Agricultural School Apolda 1899)

Introduction

Current market developments reveal how vulnerable our food systems are. Agricultural value chains are highly internationally intertwined with farm produce as well as production factors being traded to a large extent between nations (Lun et al. 2021).

Mays (*Zea mays* L.) needs phosphorus (P) to grow. Farmers try to sow the maize as early as possible in order to prolong the vegetation period and by this realize the yield potential of a variety. However, under cold soil conditions the uptake of nutrients by the roots is impeded and slowed down (Zhou et al. 2021; Moradtalab et al. 2018). Moreover, especially early developmental stages in maize were reported to determine successful crop establishment and also genotypic differences are revealed at this stage (Pedersen et al. 2022; Lu et al. 2020; Hölker et al. 2019a; Peter et al. 2009; Gordon et al. 1997). Hence, mineral starter fertilizers, in particular combinations of nitrogen (N) and phosphorus, became a common practice in maize cultivation in countries with an intensive agricultural production (Hertzberger et al. 2021; Mascagni Jr. and Boquet 1996; Teare and Wright 1990).

Status quo

On a global scale, P fertilizers were used for agricultural production in a quantity of 43,83 Mt (FAOSTAT 2019) in the year 2019. However, P resources are limited and reserves are distributed unequally across a few countries (Li et al. 2019b; Cooper et al. 2011; van Vuuren et al. 2010). Likewise, the application and nutrient balances vary substantially in different regions of the world (Vitousek et al. 2009). China, which has to provide food for around 20 % of the world’s population on only 7 % of the global arable land (Wu et al. 2021), is often taken as an example for intensive agricultural production. Average P fertilizer application rates in North China amount to over 90 kg/ha per year (Vitousek et al. 2009). At the same time, only 20 % of the applied fertilizers are eventually taken up by the plants (Bello 2021). Therefore, surpluses of P fertilizers hold the potential to be reduced by 75 % without negatively affecting Chinas food self-sufficiency (van Wesenbeeck et al. 2021). It must also be noted that phosphate efficiency of crops decreases with increasing P application (Rose and Wissuwa 2012).

In Germany, diammonium phosphate (DAP), containing nitrogen and phosphate, is the most widely used starter fertilizer in maize cultivation, which is imported to the value of over 111 million US-Dollar annually (FAOSTAT 2019). One consequence of such a supply with fertilizer inputs over the last decades are the high to very high soil P levels which are prevalent in Western Europe (Zhang et al. 2017). Another unwanted result of a high P supply are negative effects on the environment (Withers et al. 2020; Smith 2009; Liu et al. 2008). Due to this fact, the European Union has launched the Farm to Fork strategy within the framework of the EU Green Deal (European Commission 2020). Farmers are asked to reduce nutrient losses by at least 50 % until 2030 and fertilizers are aimed to be cut down by 20 %. In Germany for instance, the fertilizer ordinance restricts P to a surplus of 10 kg/ha in relation to the total amount of P removed from the field (Federal Ministry of Food and Agriculture 2017).

Fertilizer prices have been relatively stable over the last five years until 2021. During the last months, however, prices have been skyrocketing to unforeseen new price levels with no relaxation or turn in sight (Figure 1). Currently, a farmer has to pay 1100 € for one ton of DAP, which corresponds to a price increase of 122 % within one year. The same trend holds true for the fertilizers containing the single macronutrients N (calcium ammonium nitrate, CAN) and P (triple super phosphate, TSP).

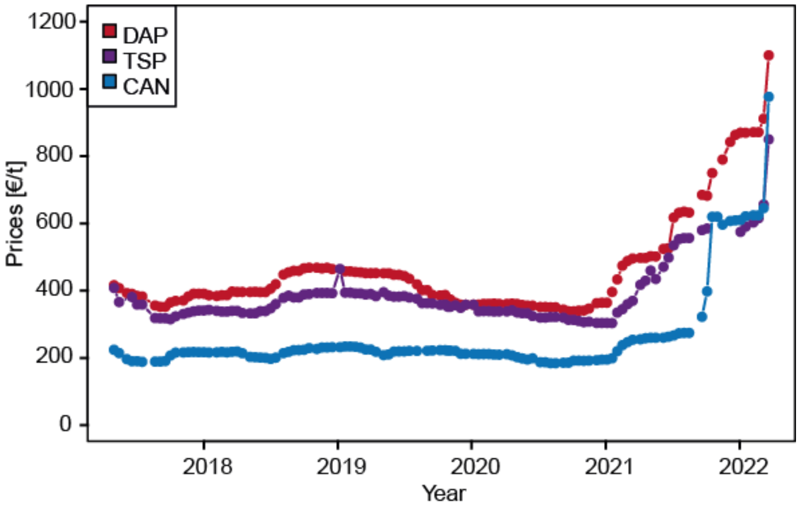

Figure 1 Development of the fertilizer prices for diammonium phosphate (DAP), triple superphosphate (TSP), and calcium ammonium nitrate (CAN) since May 2017 in Germany (data by courtesy of the State Agency for Agriculture, Food and Rural Areas (LEL), Department of Agricultural Markets and Quality Assurance).

In how far fertilizer prices have an impact on maize production, largely depends on the size and type of farm. While the current fertilizer price increase undoubtedly leads to an economization, wherever this is possible, maize cultivation areas are affected differently. In poorer countries, fertilizer use decreases as an immediate reaction to price increases and maize cultivation area, which is dependent on fertilizer application, decreases alike (Komarek et al. 2017). In highly supplied regions, as for example Germany, no major yield penalties are to be expected in the short term (Zhang et al. 2017). Therefore, the challenge in the latter case is to maintain yields through a higher nutrient efficiency. This demands plant breeders to develop and provide maize varieties, which can cope with reduced fertilizer inputs.

Taken together, it is timely to deal with phosphate efficiency in European maize production systems and investigate the topic from a plant breeding point of view. Already now some EU governments ask to postpone the ambitious goals set by the Green Deal and call for a focus on food security producing agricultural goods on all available arable land. As a matter of fact, the European Union is currently importing food on a large scale and therefore a shift of environmental damages to outside of the union should be prevented (Fuchs et al. 2020). Thus, we have to ask ourselves whether it is possible to make maize production less dependent on additional mineral fertilizer inputs, more efficient in the uptake and utilization of nutrients and therefore less vulnerable to fertilizer price rises.

P as essential plant nutrient

Plant nutrition cannot be ignored, when dealing with breeding for phosphate-efficient varieties. Justus von Liebig, a German pioneer in plant nutrition, acknowledged the importance of P as essential macronutrient already in the 19th century: if P availability is limited, plant growth is impaired (Liebig 1841). Therefore, it is of interest to know the characteristics of the different forms of P, which are to be found in plants and soil.

P in plants

P in living organisms occurs exclusively in the form of orthophosphates; it plays an essential role in the energy metabolism (e.g. adenosine triphosphate) and constitutes the backbone of DNA (Campbell and Reece 2009). At maturity, grains contain 90 % of the total P in the maize plant (Zhang et al. 2022), whereby the highest P uptake occurs around the silking stage (Ciampitti et al. 2013). While inorganic phosphorus (Pi) in the form of phosphates is highly mobile in the plants (Pedersen et al. 2022), stored in the vacuoles (Rose and Wissuwa 2012) and transported via the xylem (Victor Roch et al. 2019), P in the seed is present as an organic compound, namely phytate (IP6, Figure 2).

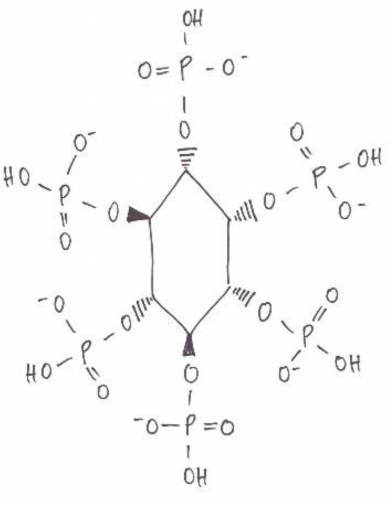

Figure 2 Structural chemical formula of phytate, an organic compound, anion of phytic acid and main storage form of P in the plants.

In mature maize seeds, phytate accounts for 50 – 80 % of total P (Elgorashi Bakhite et al. 2021). Organic phosphorus (Po) – in contrast to metals and Pi – is mainly transported via the phloem to the seeds (Palmer and Guerinot 2009). Moreover, the ratio of IP6 to total P is strongly genotype-dependent (Ragi et al. 2022) and low-phytate varieties have been successfully developed in the past due to this variation (Raboy 2002). Phytate as the phosphate storage molecule of plants will increase with elevated CO_2 levels, which are to be expected in the future (Soares et al. 2021).

P in the soil

P in the soil is extremely immobile (Bilyera et al. 2022). Its transport mainly works via diffusion and is therefore rather slow (Bello 2021; Kanno et al. 2016). In soils, we also distinguish an organic fraction and an inorganic fraction of P. Organic P usually represents 30 – 65 % of total soil P (Appelhans et al. 2021; Castagno et al. 2021). However, a large proportion of Po is locked in the form of phytate (Balaban et al. 2016; Figure 2). P is usually absorbed by minerals and therefore shows very low leaching. On the other hand, legacy P contents of soils are highly dependent on the slope and therefore soil erosion (VandenBygaart et al. 2021). Furthermore, Pi availability is mainly determined by the pH, with a neutral pH resulting in the highest availability (Joshi et al. 2021; Yu et al. 2021). Under an acidic pH, aluminum and iron compounds are formed, whereas a basic pH will lead to calcium and magnesium complexes. Thus, aluminum tolerance and phosphate efficiency are complementary breeding goals in highly weathered soils as for example prevalent in sub-Sahara Africa or South America (Leiser et al. 2014b; Magalhaes et al. 2007). P bioavailability is mainly determined by the soil conditions, but also by the plant species and plant nutritional status (Hinsinger 2001). Simply adding more P fertilizers does therefore not necessarily solve the problem of a low P availability in the soil.

Roots – the interface between plant and soil: plant reactions to P deficiency

P is taken up by the plants either directly as orthophosphates or indirectly via the mycorrhizal pathway (Ma et al. 2021; Bernardino et al. 2019; Victor Roch et al. 2019; Bovill et al. 2013). The vast majority of all plants uses the symbiosis with arbuscular mycorrhizal fungi to acquire nutrients in a more efficient way (Das et al. 2022). It is estimated that 90 % of the P requirement is covered by such symbioses (Liu et al. 2016). P deficiency can cause several phenotypic reactions such as a delay of flowering time, a reduced plant height, and lower yields (Leiser et al. 2015; Leiser et al. 2012). It seems logical that the roots as interface between soil and plants show various reactions due to insufficient P supply. As a consequence of P deficiency, plants try to maximize the surface for plant-soil interactions by enhancing arbuscular mycorrhizal fungi symbioses as one strategy or root hair growth as another strategy. Comparing these two alternatives, a 33 % higher dependency on mycorrhiza compared to root hairs was reported for maize (Ma et al. 2021). However, breeding for colonization with arbuscular mycorrhizal fungi has not proven promising as it depicts a trait with a low heritability due to a low genetic variation (Leiser et al. 2016). Therefore, root architecture traits are more promising for selection as they have been shown to be highly correlated with the above-ground plant architecture in maize (Wu et al. 2022). P deficiency will lead to the shortening of primary roots and an increase of lateral roots since P is mainly present in the soil surface (Magalhaes et al. 2018). Next to the mentioned morphological changes as reaction to P deficiency, plants also undergo physiological and biochemical adaptations. Hereby, the phosphate transporter (*PHT*) gene family plays a crucial role. *PHT* genes are mainly expressed in the root cells (Zhang et al. 2021a; Liu et al. 2016). As immediate reaction to P starvation, the Pi:H^+ Co-Transporter *PHT1* genes are differentially regulated (Nussaume et al. 2011; Nagy et al. 2006). Summing up, roots play a paramount role in Pi acquisition, which is also highlighted by the findings of a consensus quantitative trait loci (QTL) study on low P tolerance in maize that found 17 of in total 23 QTL being related to root traits (Zhang et al. 2014).

P concentration as central trait for phosphate efficiency breeding

Leiser et al. (2014a) underlined that P concentration in the grain can serve as an easy and reliable measure for phosphate efficiency indicators. Assessing the P concentration in different tissues gives us, together with the amount of dry matter, information about how much P was taken up by the plants, how it is distributed, remobilized, and eventually utilized within the plant. Hence, we usually distinguish between phosphorus uptake efficiency (PUpE) and phosphorus utilization efficiency (PUtE) to characterize the phosphate efficiency of a crop. Generally, both components are interconnected, whereby PUpE is especially relevant in P limiting environments and PUtE is more important in high P conditions (Bello 2021; Bovill et al. 2013; Rose and Wissuwa 2012). In maize, PUpE accounted for 74 % of the phenotypic variance for phosphate efficiency (Parentoni and Souza Júnior 2008). After all, P concentration as single quantity has a limited information content. However, in combination with the yield quantities, we can derive the P content as product of P concentration and yield (Li et al. 2021a; Rose et al. 2016; Azevedo et al. 2015; Leiser et al. 2015; Ciampitti and Vyn 2014; Wissuwa and Ae 2001). Like this, we capture the total amount of P being removed from the field, which is – as mentioned earlier – the decisive parameter. An important question to ask is, whether we want to breed for high or low P concentrations and whether this varies in different contexts. High P concentration in plant biomass at harvest generally indicates a high P uptake efficiency, whereas an efficient genotype should show low P concentrations as a result of successful translocation to the grains (Leiser et al. 2015; Rose et al. 2016). For the grains however, high P concentrations are generally undesired (Ludewig et al. 2019; Wang et al. 2017). Phytate, which makes up the major part of total P in the grain, absorbs micronutrients and by this strongly inhibits iron and zinc uptake by humans (Akhtar et al. 2018). At the same time, P in the grain is essential during the germination of seeds and autotrophic nutrition phase for a successful establishment of the maize plant (Doria et al. 2009; Raboy et al. 2001). Having said this, the breeding for low phytate varieties in maize has not led to significant declines in seed quality parameters or key agronomic traits (Ragi et al. 2022; Elgorashi Bakhite et al. 2021).

The last paragraphs have demonstrated the complexity of P nutrition, which we first have to be aware of when breeding for phosphate efficiency. Breeders also talk of a genotype-by-P-environment nightmare since – as elaborated above – there are so many factors that influence P availability and it is a challenge to filter out genotypic responses from the environmental noise.

Maize – one of the big three

Maize belongs with wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) to the most grown crops worldwide and is cultivated in Germany on an area of about 2.6 million hectares (German Maize Committee 2022). With the help of molecular data the introduction of maize into Europe can be reconstructed, revealing several separate introduction events right after the discovery of the New World (Rebourg et al. 2003). These independent introductions allowed for the adaptation of maize to different climates (Tenaillon and Charcosset 2011). Nowadays, maize is very widely adapted to different day lengths and temperatures (Liu et al. 2015). Transfer of elite lines within the same maturity groups is common practice in internationally operating maize breeding companies in order to broaden genetic pools (Steinhoff et al. 2012).

Furthermore, maize breeding history is the favorite example to demonstrate the successful exploitation of heterosis (Duvick 2005; Hallauer 1999). The concept of modern hybrid breeding was independently developed by George Harrison Shull (Shull 1909) and Edward Murray East in the beginning of the 20th century. Hybrid seed production developed from double cross hybrids to single cross hybrids due to an improved yield potential of the parental inbred lines (Duvick 2001). As a basis, different heterotic pools have been established (e.g. Unterseer et al. 2016). The different kernel textures are one characteristic to assign individuals to heterotic pools. In Central Europe, Flint × Dent crosses represent the common breeding scheme for early to mid-late maturing hybrids (Barrière et al. 2006). Breeding progress in both pools over the last decades has been tremendous, also for the European Flint pool, which generally shows a narrower genetic basis compared to the Dent pool (Hölker et al. 2019b; Rebourg et al. 2003).

Low temperatures as limiting factor

As mentioned before, maize is very sensitive to cold temperatures especially in early growth stages and farmers are sowing their maize as early as possible to maximize the vegetation period and attempt to avoid other stresses (Yi et al. 2021). Therefore, cold tolerance remains an important breeding goal with no genes detected to predict for it in maize (Frascaroli and Revilla 2018). Studies on frost tolerance in different crops also confirmed a complex architecture of this trait, which is determined by multiple QTL (e.g. Shakiba et al. 2017; Liu et al. 2014; Galiba et al. 2009). Cold tolerance in maize is usually measured as emergence and seedling growth under cold temperatures (Revilla et al. 2000). In Europe, the selection of an ‘early allele’ along the northern Flint migration route took place while in warm environments this allele was eliminated due to its negative effects on plant size and yield (Tenaillon and Charcosset 2011). This is why individuals from the Flint pool show a better adaptation to cold conditions (Yi et al. 2021), which are prevalent during the sowing season in Central Europe. The maize field season in Germany usually lasts less than 6 months and focus in breeding activities is due to the abovementioned reasons also given to a good crop establishment (Figure 3, activity [3], [4] and [5]).

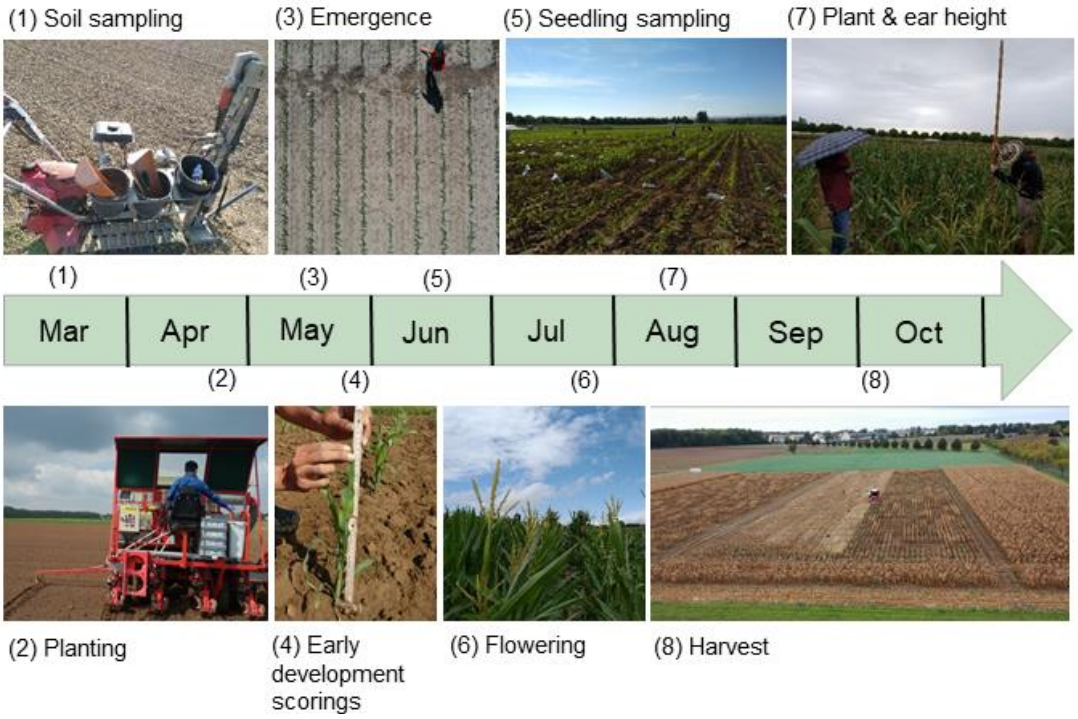

Figure 3 Field activities during the growing season in the maize nursery. Unmanned aerial vehicle images were taken by Alice-J. Reineke.

A new era: the doubled haploid technology

The advantages of developing homozygous inbred lines in an instantaneous way were soon identified but first faced several difficulties and thus were not implemented in breeding programs (Hallauer et al. 1988). For this purpose, haploids are induced, which in a subsequent step are doubled to derive completely homozygous lines (Geiger and Gordillo 2009). But only with the discovery and advent of higher haploid induction rates, maize breeding experienced a revolution with doubled haploid (DH) lines being developed in only two to three generations (Doubled haploid technology in maize breeding: theory and practice 2012). DH lines offer several benefits, above all higher selection gains per unit time by means of simplified logistics, rapid fulfillment of distinctness, uniformity and stability criteria, and greater efficiency and precision of selection in combination with the use of molecular markers (Geiger and Gordillo 2009; Röber et al. 2005). Nowadays, haploid induction rates with an inducer line used as pollen parent of on average 8 – 10 % are realized and identification of successful induction is facilitated by coloration markers or oil kernel content. Doubling of the chromosomal number is usually performed with colchicine treatment. As a last step, DH lines are recovered that can then be used in further breeding. The doubled haploid technology unfolds its full potential in combination with marker-assisted selection and year-round nurseries (Doubled haploid technology in maize breeding: theory and practice 2012). Nowadays, *in vivo* doubled haploid production is part of most commercial breeding programs of maize.

Landraces – potential gold mines?

Landraces represent a huge genetic variation (Figure 4). More than 10 000 accessions of maize are classified as populations and stored in European seedbanks from Austria to Yugoslavia (Lipman et al. 1997, Table 1). Unfortunately, with the overwhelming success of hybrids, landrace populations disappeared from the breeders' radar. Producing DH line libraries from genetic resources was proposed as an approach to broaden the genetic basis of the Flint pool, as they offer rich genetic diversity in an adapted background (Würschum et al. 2022; Brauner et al. 2019; Böhm et al. 2017; Melchinger et al. 2017; Wilde et al. 2010). In doing so, heterogeneous landrace populations composed of heterozygous genotypes are purged from their high genetic load and thereby rapidly made available for breeding (Melchinger et al. 2017). It has to be considered, however, that the costs to produce DHs from landraces are three times higher than obtaining to DHs from elite material (Böhm et al. 2017). Having said this, if compared to maintenance costs of seed banks, the costs for producing DH libraries only moderately exceed these (Melchinger et al. 2017). Nevertheless, resources must be allocated effectively by first choosing the most promising landraces for DH production. Subsequently, the best performing DH lines from landraces should be identified for further breeding (Böhm et al. 2017). Testcross performance of DH lines from landraces suggest that they cannot be used as hybrid parents immediately but have to undergo backcrossing or other pre-breeding beforehand (Wilde et al. 2010). However, if landraces are carefully chosen before DH production, testcross performance of DH lines from landraces showed comparable results to elite lines (Brauner et al. 2019). Since the genetic variance within landraces was reported to be by far higher than among different landrace populations, it is recommended to rather focus on a few landraces and sample them intensively than producing a few DHs from many different landraces (Böhm et al. 2017). The mining of landrace haplotypes is promising since more than a quarter of haplotypes could not be found in elite breeding lines (Mayer et al. 2020). Moreover, landraces are perfectly suited to be used in genome-wide association studies (GWAS). Through their rapid decay of linkage disequilibrium, they allow for a high mapping resolution (Würschum et al. 2022).

The allelic diversity hidden in landraces may assist the breeding for cold tolerance and the crop establishment at early growth stages by being made available as DH libraries (Hölker et al. 2019a; Mayer et al. 2020). Other traits for which a narrow genetic basis exists in breeding material, like for instance phosphate efficiency, could also benefit from retrieved genetic variation of maize landraces.

Figure 4 Phenomena observed in a diverse set of maize doubled haploid lines from landraces. Leaf colorations, tillering, flower abnormalities, lack of resistance to diseases and lodging are likely to be encountered.

Maize breeding in Europe is dominated by a few large seed companies. The improvement of phosphate efficiency has not yet been in the focus of their breeding activities. Due to root system differences, maize generally has a lower phosphate efficiency compared to wheat and rice (Yu et al. 2021). In particular, the supply of maize with P during early growth stages is relevant, when root systems are not yet fully developed and the switch to heterotrophic nutrition takes place (Lu et al. 2020). Having seen the many factors determining P availability for maize plants, the most important factor influencing the maize yield response to P and therefore phosphate efficiency is the variety (Yan et al. 2021). Hence, it seems reasonable, particularly in the setting of sharply rising fertilizer prices, to put a focus on breeding for nutrient-efficient maize varieties.

Objectives of this thesis

The goal of this thesis was to investigate phosphate efficiency in maize in a European context. In particular, we wanted to answer the following research questions:

(1) Which genetic variation exists in commercial maize hybrids to make them less dependent on extra P fertilizer inputs?
(2) How can traits for phosphate efficiency be cost- and time-efficiently predicted in breeding programs?
(3) Do doubled haploid lines from European landraces harbor valuable genetic variation for phosphate efficiency traits?
(4) What should breeding programs focusing on phosphate efficiency take into consideration?

Publications

Optimizing the P balance: How do modern maize hybrids react to different starter fertilizers?

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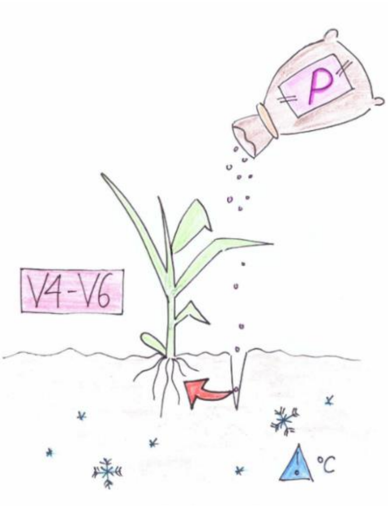

Figure 5 Graphical excerpt of publication 1. Different starter fertilizers were applied to commercial maize hybrids. Mainly the early development of maize, namely the four- to six-leaf stage (V4 – V6), was affected by starter fertilizers. We observed a negligible G×P interaction, but a strong environmental effect, especially with regard to cold and wet conditions.

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RESEARCH ARTICLE

Optimizing the P balance: How do modern maize hybrids react to different starter fertilizers?

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Abstract

Phosphorus (P) is an essential macronutrient for plants, but also a limited resource worldwide. Strict regulations for fertilizer applications in the European Union are a consequence of the negative environmental effects in case of improper use. Maize is typically grown with the application of P starter fertilizer, which, however, might be reduced or even omitted if suitable varieties were available. This study was performed with the 20 commercially most important maize hybrids in Germany evaluated in multi-location field trials with the aim to investigate the potential to breed for high-performing maize hybrids under reduced P starter fertilizer. At the core location, three starter fertilizers with either phosphate (triple superphosphate, TSP), ammonium nitrate (calcium ammonium nitrate, CAN), or a combination of ammonium and phosphate (diammonium phosphate, DAP) were evaluated relative to a control and traits from youth development to grain yield were assessed. Significant differences were mainly observed for the DAP starter fertilizer, which was also reflected in a yield increase of on average +0.67 t/ha (+5.34%) compared to the control. Correlations among the investigated traits varied with starter fertilizer, but the general trends remained. As expected, grain yield was negatively correlated with grain P concentration, likely due to a dilution effect. Importantly, the genotype-by-starter fertilizer interaction was always non-significant in the multi-location analysis. This indicates that best performing genotypes can be identified irrespective of the starter fertilizer. Taken together, our results provide valuable insights regarding the potential to reduce starter fertilizers in maize cultivation as well as for breeding maize for P efficiency under well-supplied conditions.

OPEN ACCESS

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1 | Introduction

Phosphorus (P) is a globally limited reserve [1]. There are varying predictions of how long phosphate rock reserves will last, but most studies anticipate a time frame between 100 and

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400 years [2]. Moreover, P reserves are unevenly distributed across the globe [3] and often contaminated with heavy metals [3, 4]. In living organisms, phosphorus always occurs in the form of phosphates (P_i) and plays an essential role as a component of the DNA, cell membranes, and coenzymes as well as in the energy transfer processes of cells. Thus, P is deemed one of the most important macronutrients for plants [5].

Maize (*Zea mays* L.) is one of the three major staple foods worldwide with a cultivated area of around 194 million hectares in 2018 [6], of which Germany grows more than 2.6 million ha, primarily for silage usage [7]. It is common agricultural practice since the 1980s in industrialized countries to apply starter fertilizers in maize cultivation, specifically combinations of ammonium and phosphate [8–10]. Germany, for instance, used over 90.8 Mt of P fertilizer in 2017 [11]. However, the known negative environmental effects [12, 13] due to an inappropriate use of fertilizers—in its worst form, the eutrophication of surface water by run-off and leaching into drainages or deeper soil layers–have gained increasing attention in the public perception and the shaping of agricultural policies [14, 15]. Recently, the Farm to Fork Strategy of the European Union was released, which aims for a reduction of nitrogen and phosphate fertilizers of 20% by 2030 [16]. In Germany, a new fertilizer ordinance has been introduced, dictating the documentation of nitrogen and phosphate fertilizer usage for the whole farm; fertilizer applications are restricted depending on the overall nutrient removal of each crop [17]. Several studies have shown that P in Europe is sufficiently to abundantly available on agricultural lands [18]. This holds specifically true for agricultural businesses that have a surplus of organic fertilizers available, namely livestock- and biogas-based farms [19, 20]. In many cases however, the abundant P is fixed by minerals and therefore not fully available for plants [21].

Taking these facts together, it now appears timely and reasonable to breed for an optimized use of phosphate fertilizers in maize in order to achieve an improved ecological footprint. Phosphate-use-efficiency (PUE) is classically defined either as high P concentrations of the harvested organs due to a higher P *uptake* of the roots or as increased yields per given unit P due to a better internal *utilization* of the available P [22–24]. In previous studies, it was shown that the traits early vigor, early-season plant height, flowering, and yield react to P deficiency in sorghum and can thus be considered as P-sensitive traits [23]. Conversely, starter fertilization in maize may lead to an increase in grain yield of 4.5% in comparison with broadcast fertilization [25].

Nevertheless, little is known about the reaction of modern maize hybrid varieties to different starter fertilizers. We therefore conducted a field trial with 20 modern maize hybrids evaluated at five locations within Germany under a control (Co) and three different starter fertilizers treatments, i.e. phosphate (triple superphosphate, TSP), ammonium nitrate (calcium ammonium nitrate, CAN), or a combination of ammonium and phosphate (diammonium phosphate, DAP). In particular, our objectives were to (i) assess the variation in the response to different P starter fertilizers in maize cultivation, (ii) evaluate the genotype-by-starter fertilizer interaction, (iii) identify high-yielding and P-stable maize hybrids, and (iv) draw conclusions for maize breeding.

2 | Material and methods

2.1 | Plant material

For this study, the 20 commercially most important maize hybrids in Germany were chosen. They belong to eight breeding companies and the vast majority represents the mid-early maturity group (FAO 200–270). All varieties are suited for grain or corn-cob-mix utilization and were harvested as grain maize. Moreover, all seeds were treated in the standard way of each company (S1 Table).

Table 1. Description of the locations.

	Altitude [m ASL]	Ø Temp.* [˚C]	Ø Precip.* [mm]	Soil type	pH	P_2O_5 [mg/100g soil]	P [mg/100g soil]	Classification of P availability**
Hohenheim (HOH)	402	10.6	857	Silty Loam	6.79	21.1	9.2	D
Eckartsweier (EWE)	142	11.7	783	Clayey Loam	6.54	19.2	7.7	D
Dettingen (DET)	561	9.1	661	Clayey Loam	7.33	52	20.5	E
Einbeck (EIN)	124	8.7	679	Clayey Loam	6.85	19.5	7.7	D
Saerbeck (SAB)	56	9.3	789	Strongly Loamy Sand	5.75	24.7	10.7	D

Including altitude, weather data (mean temperature, mean precipitation), soil type, and pH. According to the P status, the 'Classification of P availability' of the soils can range from A (very low) to E level (very high).

* Data for locations in Baden-Württemberg retrieved from <www.wetter-bw.de>, for locations outside of Baden-Württemberg retrieved from <climate-data.org>

** According to VDLUFA-P-content-classes (A = very low, E = very high) defined by the Association of German Agricultural Analytic and Research Institutes (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten).

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2.2 | Field trial

We applied four treatments, i.e. a control (Co: 0% N/ 0% P), and the three different starter fertilizers triple superphosphate (TSP: 0% N/ 20% P), calcium ammonium nitrate (CAN: 26% N/ 0% P), and diammonium phosphate (DAP: 18% N/ 20% P). The field design was laid out as an alpha-lattice (5×4) using the software CycdesigN [26]. Genotypes were replicated twice per starter fertilizer treatment and the trial was conducted at five different locations. Hohenheim served as core location with the control and all three different starter fertilizers. All other locations comprised the control and either TSP or DAP, resulting in total in three locations with TSP, three with DAP, and one with CAN (S2 Table). The weather data including soil temperatures in Hohenheim (S1 Fig) for the calendar year 2019 [27] was characterized by an extraordinary cold phase in May right after sowing the trial, which led to a delayed emergence. The altitude of the field locations ranged from 56 to 561 m above sea level, the average temperatures varied from 8.7 to 11.7˚C, and the average annual precipitation amounted to 661 to 857 mm in 2019. All locations were thoroughly characterized regarding their soil properties and phosphorus status before the trial started (Table 1). The P status of the soils was analyzed according to the method for plant available P by the Association of German Agricultural Analytic and Research Institutes (VDLUFA). Phosphates were extracted with 100 mL solution of calcium acetate, calcium lactate and acetic acid buffered to pH 4.1 from 5 g air-dry soil followed by a photometric determination [28]. It is crucial to notice that all investigated soils showed levels of plant available P between 7.7 and 20.5 mg P/100g soil, therefore showing high to very high P availability according to the P-content-classes defined by the VDLUFA [29]. Overall, best agricultural management practice was followed, adapted to the individual agronomic demands of each location (e.g. Trichogramma treatment, herbicide application, etc.). The field season across locations ranged from 23rd of April to 29th of October 2019, sowing densities ranged from 8.8 to 10 plants/m^2, and plot sizes from 7.5 to 18 m^2 (for the latter only the middle rows were considered for grain harvest) according to the local standard practice (S3 Table).

2.3 | Phenotypic data

During the field season 2019, the following traits were assessed: plant height at up to four different developmental stages (PH, cm), ear height (EH, cm), days to anthesis (DTA, days after sowing, abbreviated as DAS), days to silking (DTS, days after sowing), anthesis-silking-interval

(ASI, days), grain dry matter content (GDM, %), grain yield (GY, t/ha), P grain concentration (Pconc, mg/kg), and P grain content (Pcont, kg/ha; calculated as GY*Pconc/1000). Details of how the traits were scored are provided in S4 Table. In case a trait was not measured at a location, the data were treated as not available (NA).

2.4 | Statistical analyses

First, we checked the quality of the phenotypic data of all traits on the single location level. The statistical model for this analysis was:

$$y_{ij} = \mu + g_i + r_j + \varepsilon_{ij}, \tag{1}$$

where y_{ij} stands for the trait value of the i-th genotype in the j-th replicate; μ denotes the overall mean, g_i the effect of the i-th genotype, r_j the effect of the j-th replicate and ε_{ij} the residual. Outlier detection was performed on the single location level applying the Bonferroni-Holm method [30].

In a second step, the analysis was performed across locations and the mixed model of the single location analysis was extended to the full model:

$$y_{ijkl} = \mu + t_i + g_j + l_k + (tg)_{ij} + (tl)_{ik} + (gl)_{jk} + (tgl)_{ijk} + r_{ikl} + \varepsilon_{ijkl} \tag{2}$$

where y_{ijkl} stands for the trait value of the j-th genotype at the k-th location in the l-th replicate grown under the i-th starter fertilizer; μ denotes the overall mean, t_i the effect of the i-th fertilizer treatment, g_j the effect of the j-th genotype, l_k the effect of the k-th location, $(tg)_{ij}$, $(tl)_{ik}$, $(gl)_{jk}$ represent the corresponding two-way interaction terms, $(tgl)_{ijk}$ the three-way interaction term, r_{ikl} refers to the replication nested within the location and each starter fertilizer, and ε_{ijkl} is the residual term. As for the single location analysis, all factors were treated as random to estimate the variance components except for the general mean and the starter fertilizer treatment which entered the model as a fixed factor for calculations across starter fertilizers. Significance of variance components was tested by likelihood ratio tests. Repeatabilities (r^2) and broad-sense heritabilities (H^2) respectively were calculated after the concept of the generalized heritability measure [31, 32] with $H^2 = 1 - A_{tt}/(2\sigma_g^2)$, where H^2 denotes the generalized heritability, A_{tt} the average pairwise prediction error variance for the genotypic term, and σ_g^2 the genotypic variance estimate.

Best Linear Unbiased Estimates (BLUEs) were obtained for each of the investigated 20 hybrid varieties by considering the factor genotype as a fixed effect in the mixed model. All subsequent analyses were based on these BLUEs. Statistical analyses were performed with RStudio [33] and mixed model analyses were performed with ASReml-R [34]. In addition, the R-packages 'asremlPlus' [35] served for the calculation of information criteria for model selection, 'agricolae' [36] for the performance of significance tests, and 'multtest' [37] for outlier detection. Under R version 3.6.2 the R-packages 'ggpubr' [38], 'gplots' [39], and 'qgraph' [40] were used to produce plots.

3 | Results

3.1 | Response of traits to different starter fertilizers

The field trial underlying this study was based on five locations. Importantly, these can all be classified as having a high to very high P availability (Table 1). For all investigated traits, medium to very high repeatabilities were observed on the single location level. The lowest repeatabilities were found for grain yield with a minimum of 0.35, whereas grain dry matter content showed the highest values with a maximum of 0.98 (S5 Table). The phenotypic distributions and the mean trait values revealed that if there was an effect of the starter fertilizer, it was usually the DAP treatment that exhibited this effect (Fig 1 and S2 Fig). Regarding the early plant height measurements, the control always showed the lowest mean, but only the DAP treatment resulted at youth stage in significantly taller plants than the control (Fig 1a). The response observed for plant height illustrates that the youth developmental stages are generally enhanced by the application of starter fertilizers. However, these differences diminished in the course of the field season and were not significant any more for the final plant height measurement. Nevertheless, ear height measurements resulted in significantly different means depending on the starter fertilizer (S2 Fig). The anthesis-silking-interval shortened from a mean of 0.65 days in the control and TSP to a mean of 0.40 days in the DAP treatment. These differences were statistically non-significant, but it must be noted that the ASI was very narrow for all 20 hybrids and only ranged between -1 and 4 days. Similarly, grain dry matter content was slightly higher in the DAP treatment with a mean of 69.40% compared to 68.15% in the control, indicating a faster maturity process in the treatments with starter fertilizer. These trends, even though they did not lead to significant differences, are in agreement with the significant differences observed for male and female flowering (S2 Fig). The DAP-fertilized varieties flowered significantly (p-value < 0.05) earlier (mean DTA = 83.03 DAS; mean DTS = 83.43 DAS) than the control (mean DTA = 84.80 DAS; mean DTS = 85.45 DAS), and also the TSP treatment (mean DTA = 83.75 DAS; mean DTS = 84.40 DAS) flowered approximately one day earlier than the control. For grain yield, the DAP treatment once again contrasted with the other treatments, yielding on average 13.21 t/ha, while the control showed a mean of 12.55 t/ha. In accordance with the results for grain yield, the highest P content was found for DAP with a mean of 31.61 kg/ha. P content showed only a significant difference (p-value < 0.05) between the DAP and CAN (mean Pcont = 29.60 kg/ha) starter fertilizer treatments. The P concentration of the grains, by contrast, showed no significant differences among the four treatments (S2 Fig).

3.2 | Relationships among traits dependent on the starter fertilization

The network plots visualized the correlations among the investigated traits dependent on the starter fertilizer (Fig 2). While there were differences, the general patterns remained the same. Grain yield, for instance, was always negatively correlated with P concentration, which can probably be attributed to the effect of dilution. Independent of the starter fertilizer, the early plant heights PH1, PH2, and PH3 (measured 53, 59, and 63 DAS, respectively) were closely related ($0.75 < r < 0.95$; p-values <0.01) but are no predictor for the final plant height (measured 94 DAS), nor grain yield. Another consistent triangle observed throughout the different treatments was the highly positive correlation between the male and female flowering times ($r > 0.9$, p-values < 0.001), which were always strongly negatively ($r > -0.87$, p-values < 0.01) correlated with grain dry matter (S3 Fig). Moreover, there was a significant positive association between the anthesis-silking-interval and the final plant height as well as between the P grain concentration and the P grain content.

We further analyzed the relationships between the maize kernel content of 16 chemical elements in the Co, TSP, and DAP treatment of the core location Hohenheim (S4 Fig). This revealed close positive correlations of phosphorus with magnesium, manganese, potassium, sulfur, and zinc.

3.3 | Identification of P sensitive and P stable genotypes across multiple locations

Having observed an effect of the starter fertilizer on some traits, the question arises whether the overall ranking of the varieties changes, i.e. whether there is a genotype-by-starter fertilizer

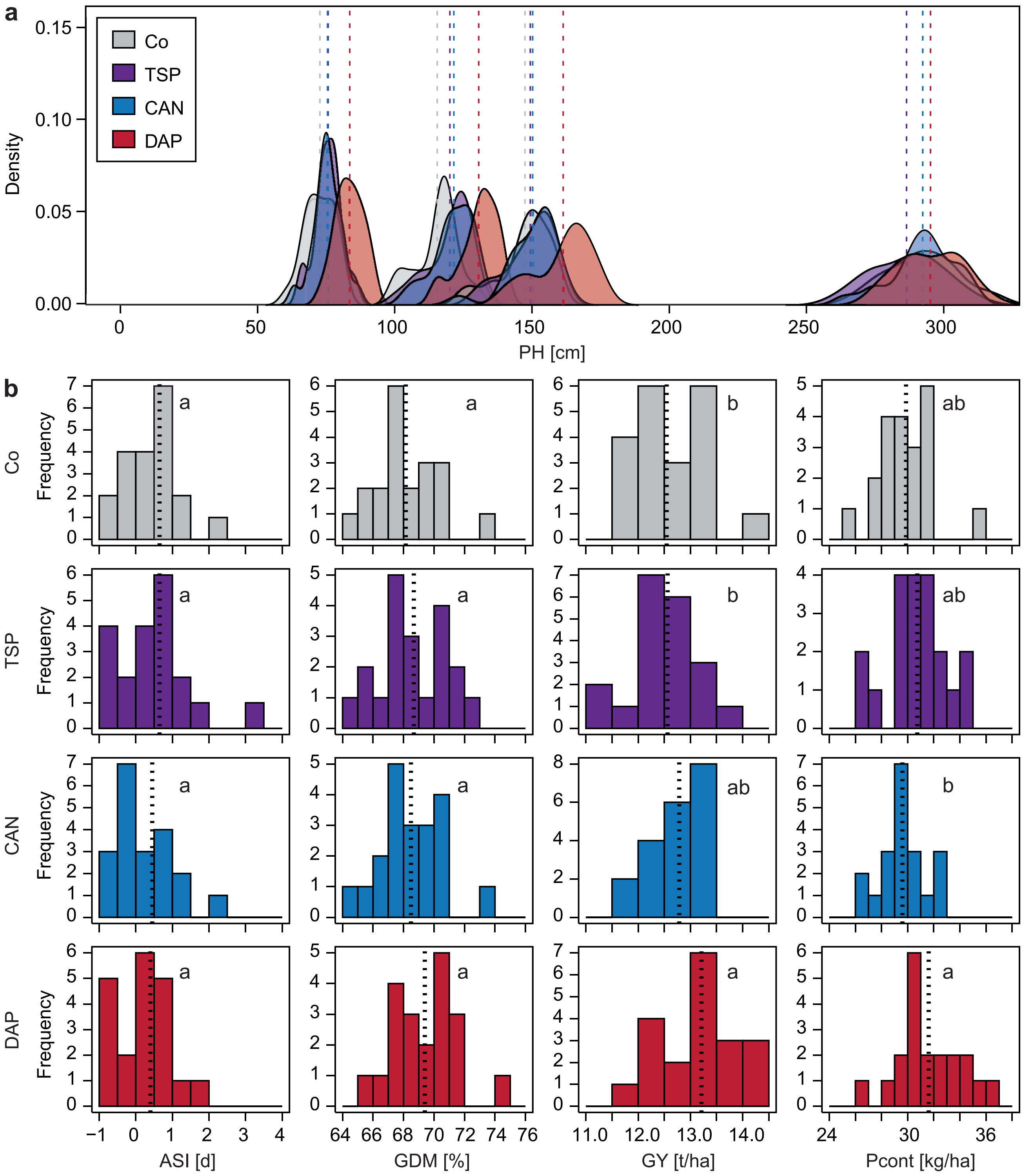

Fig 1. Response of different traits to starter fertilizers. Control (Co, grey), triple superphosphate (TSP, purple), calcium ammonium nitrate (CAN, blue), diammonium phosphate (DAP, red). (a) Density plots of plant height distributions at four different time points (PH1, PH2, PH3, PHfinal). (b) Histograms of anthesis-silking-interval (ASI), grain dry matter (GDM), grain yield (GY), and P content (Pcont). Different letters indicate significant (p-value < 0.05) differences between starter fertilizer means.

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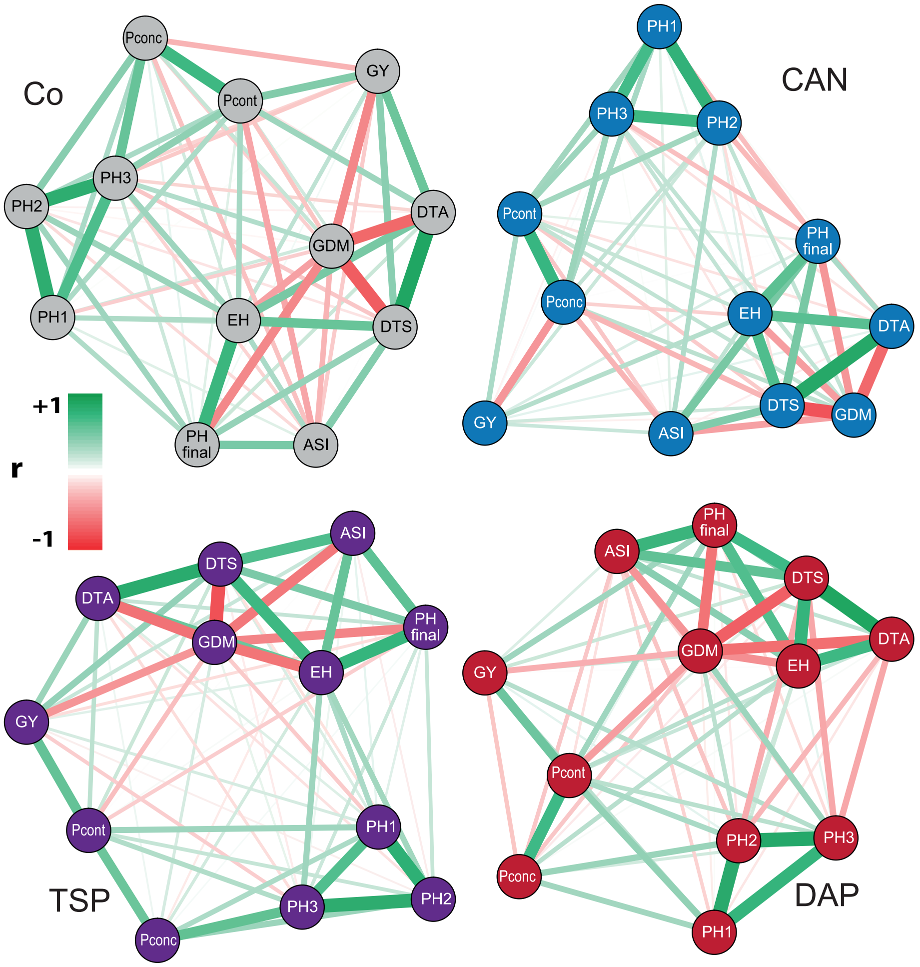

Fig 2. Associations among the investigated traits dependent on the starter fertilizer. Control (Co, grey), triple superphosphate (TSP, purple), calcium ammonium nitrate (CAN, blue), diammonium phosphate (DAP, red). Network plots showing anthesis-silking-interval (ASI), days to anthesis (DTA), days to silking (DTS), ear height (EH), grain dry matter content (GDM), grain yield (GY), P grain concentration (Pconc), P grain content (Pcont), and plant heights (PH1, PH2, PH3, PHfinal). Positive Pearson correlations (r) are indicated in green, negative Pearson correlations in red.

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interaction. This knowledge is essential for the choice of variety by the farmer but also to choose appropriate conditions for genotype selection in breeding. The rank changes across the four different starter fertilizer treatments in Hohenheim demonstrated a rather parallel shift of the performance for grain yield and P content (Fig 3). The genotype-by-treatment interaction was non-significant for grain yield (p-value = 0.46). Thus, the best performing varieties under starter fertilizer application tend to be also among the best performing varieties in the control. Likewise, the trait P content signifying the removal of P from the field showed no significant genotype-by-treatment interaction (p-value = 0.30).

This observation, made for the core location Hohenheim, was confirmed in the series across locations, for which the genotype-by-starter fertilizer interaction in the analysis of variance was never significant for any observed trait (S6 Table). The heritabilities in the series were very high except for grain yield and the trait P content derived from it. This is likely due to the highly quantitative nature of grain yield, the rather small genotypic variation in this elite material and the observed strong genotype-by-location interaction. Again, the differentiation

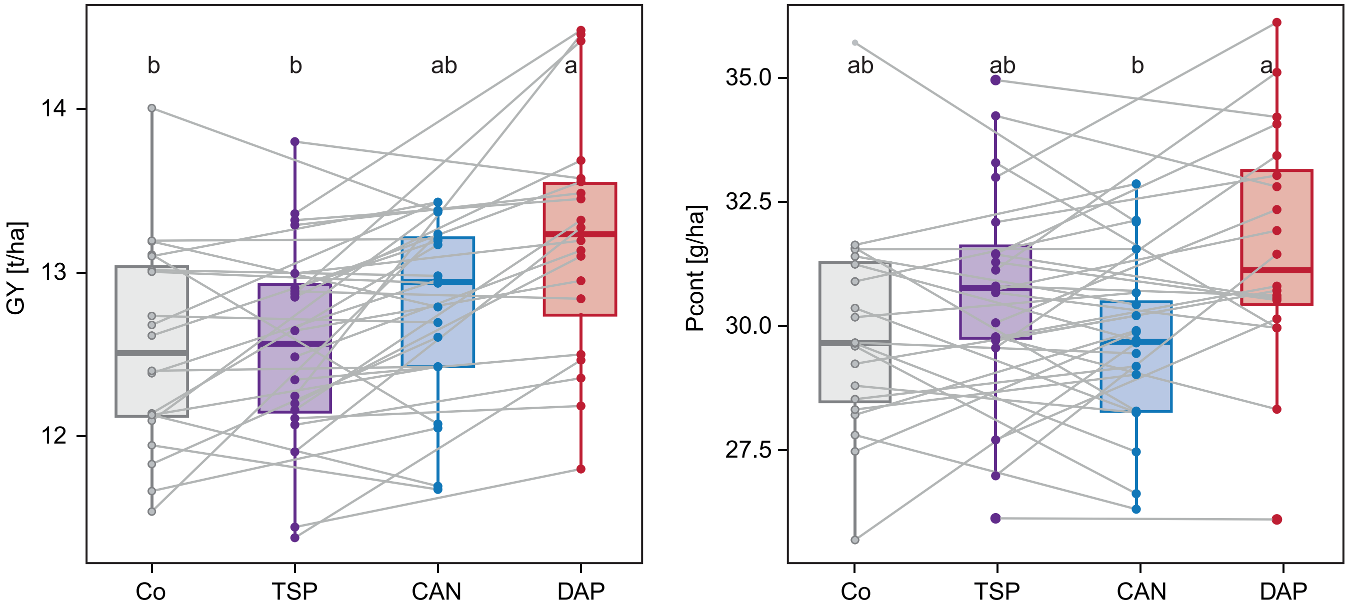

Fig 3. Visualization of the genotype-by-starter fertilizer interaction. Boxplots for grain yield (GY) and P grain content (Pcont) by starter fertilizers: control (Co, grey), triple superphosphate (TSP, purple), calcium ammonium nitrate (CAN, blue), diammonium phosphate (DAP, red). Interaction terms are visualized by connecting trait values of the same varieties with a grey line. Note that for the sake of clarity lines are only drawn between every other treatment. Different letters indicate significant (p-value < 0.05) differences between starter fertilizers means.

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was more pronounced in the comparison between DAP and the control than between TSP and the control.

Interestingly, the origin of the variety in the sense of the eight different breeding companies, did not lead to a consistently similar behavior with regard to the investigated traits under different starter fertilizers (S5 Fig). From a practical farming point of view, the most interesting question is how the different varieties perform with regard to grain yield under control and starter fertilizer conditions. Therefore, relative grain yields under TSP or DAP starter fertilizer were plotted against the relative grain yield of the control for each location (Fig 4). Varieties in quadrant I (highlighted upper right) of the plot can be defined as stably above- average-yielding P-utilizers, whereas varieties in quadrant III (bottom left) are relatively low-yielding independent of their P-supply. Varieties located in the quadrants II and IV can be considered P-sensitive genotypes, as they will show above-average yields with starter fertilizer but not in the control or vice versa, respectively. In line with the small genotype-by-treatment interaction, most varieties showed either below-average or above-average yield performance no matter which starter fertilizer they were grown under. By comparing the relative performance of all varieties in each treatment-location-combination, we identified the consistently best varieties. For the TSP-series, three varieties were in quadrant I at each location (AGROPOLIS_AM, AMAVERITAS_AM, WALTERINIO_KWS) and for the DAP-series also three varieties (AGROPOLIS_AM, FIGARO_KWS, SY_TALISMAN). Only the variety AGROPOLIS_AM was in this high-yielding quadrant at each location-treatment combination.

From a breeding point of view, it is also interesting to identify the most P-independent genotypes. While the analysis of the relative performance in the control and the starter fertilizer treatments already provided some indication to this, genotypes may be above average for both treatments, but still show a substantial reduction in grain yield when the starter fertilizer is omitted. The most interesting candidates are those showing the least reduction between starter fertilizer and control, while at the same time having a high yield. We therefore analyzed

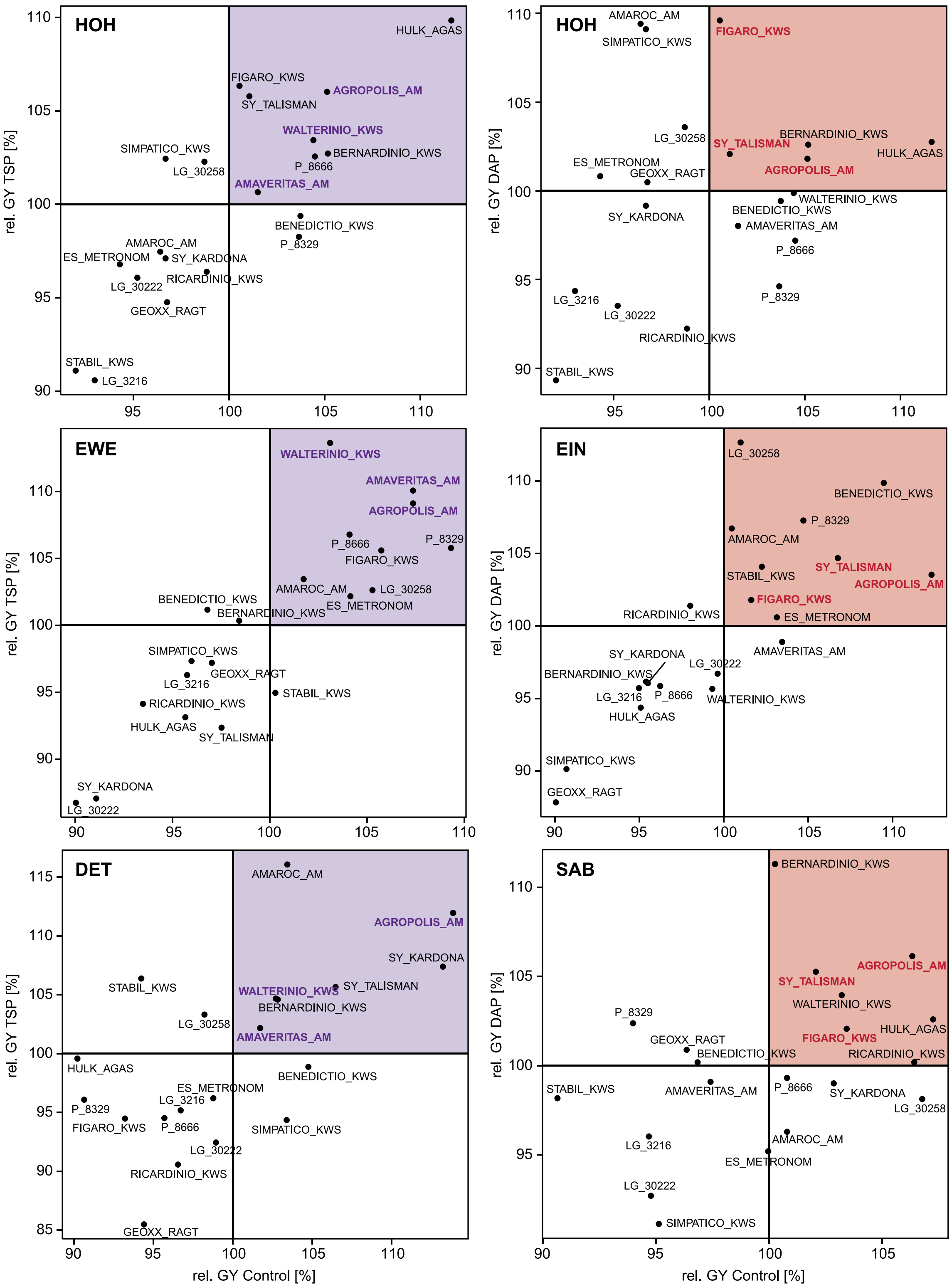

Fig 4. Scatterplots of each starter fertilizer-location combination. Relative grain yield (rel. GY [%]) in the control vs. triple superphosphate (TSP, purple) or vs. diammonium phosphate (DAP, red), respectively. Quadrants are counted starting from the highlighted one as I (stably above- average-yielding P-utilizers) in an anti-clockwise manner to IV (II and IV indicate P-sensitivity). The varieties which in all three locations were located in Quadrant I are highlighted in bold and color. Locations are abbreviated as follows: Hohenheim (HOH), Eckartsweier (EWE), Dettingen (DET), Einbeck (EIN), and Saerbeck (SAB).

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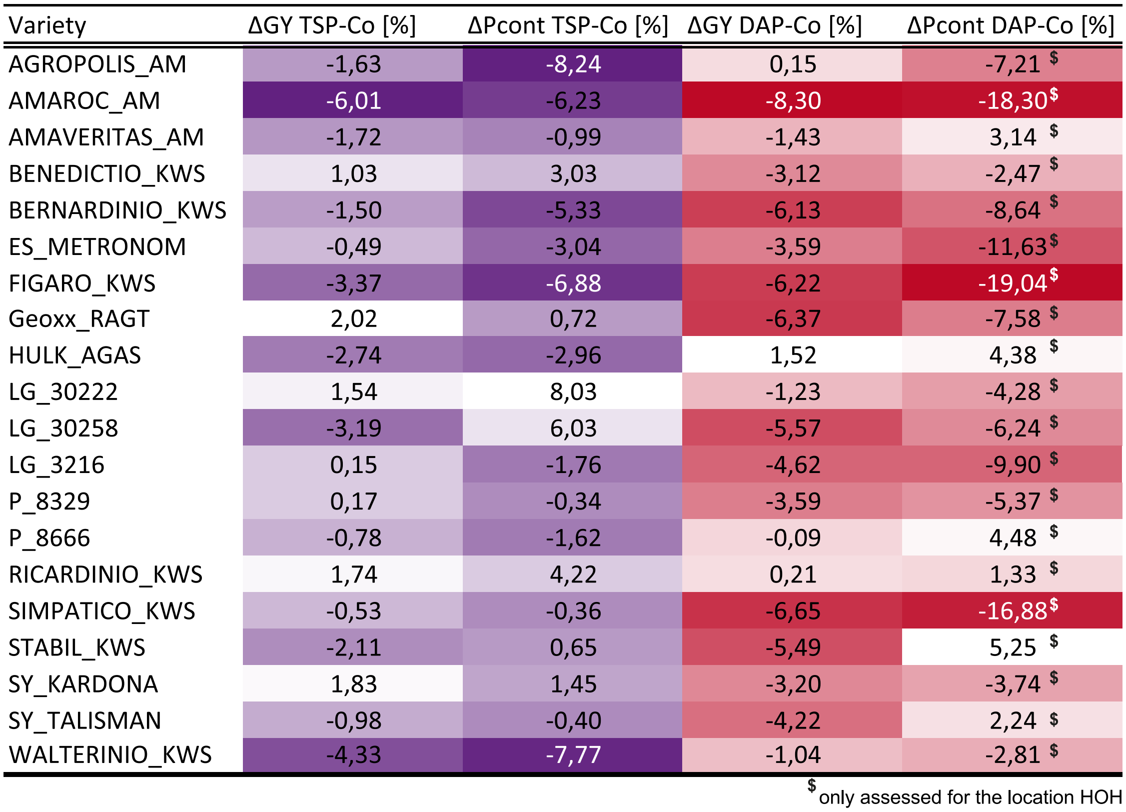

Variety	ΔGY TSP-Co [%]	ΔPcont TSP-Co [%]	ΔGY DAP-Co [%]	ΔPcont DAP-Co [%]
AGROPOLIS_AM	-1,63	-8,24	0,15	-7,21 $^{\$}$
AMAROC_AM	-6,01	-6,23	-8,30	-18,30$^{\$}$
AMAVERITAS_AM	-1,72	-0,99	-1,43	3,14 $^{\$}$
BENEDICTIO_KWS	1,03	3,03	-3,12	-2,47 $^{\$}$
BERNARDINIO_KWS	-1,50	-5,33	-6,13	-8,64 $^{\$}$
ES_METRONOM	-0,49	-3,04	-3,59	-11,63$^{\$}$
FIGARO_KWS	-3,37	-6,88	-6,22	-19,04$^{\$}$
Geoxx_RAGT	2,02	0,72	-6,37	-7,58 $^{\$}$
HULK_AGAS	-2,74	-2,96	1,52	4,38 $^{\$}$
LG_30222	1,54	8,03	-1,23	-4,28 $^{\$}$
LG_30258	-3,19	6,03	-5,57	-6,24 $^{\$}$
LG_3216	0,15	-1,76	-4,62	-9,90 $^{\$}$
P_8329	0,17	-0,34	-3,59	-5,37 $^{\$}$
P_8666	-0,78	-1,62	-0,09	4,48 $^{\$}$
RICARDINIO_KWS	1,74	4,22	0,21	1,33 $^{\$}$
SIMPATICO_KWS	-0,53	-0,36	-6,65	-16,88$^{\$}$
STABIL_KWS	-2,11	0,65	-5,49	5,25 $^{\$}$
SY_KARDONA	1,83	1,45	-3,20	-3,74 $^{\$}$
SY_TALISMAN	-0,98	-0,40	-4,22	2,24 $^{\$}$
WALTERINIO_KWS	-4,33	-7,77	-1,04	-2,81 $^{\$}$

$^{\$}$only assessed for the location HOH

Fig 5. Relative differences for each hybrid variety. Grain yield (GY) and P content (Pcont) are based on best linear unbiased estimators (BLUEs) across locations between the fertilized treatment (purple: triple superphosphate (TSP); red: diammonium phosphate (DAP)) compared to the control. The darker the coloration, the stronger the reduction.

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for each variety the reduction in grain yield and P concentration between the DAP or TSP starter fertilizer and the control across locations. This revealed the trend that if grain yield is strongly reduced without starter fertilizer, this generally goes in line with a reduced P content (Fig 5), while the P concentration does not significantly (p-value < 0.05) differ among the starter fertilizer treatments. Also, varieties that performed consistently above-average, like for instance AGROPOLIS_AM, can nonetheless show proportionally strong reductions without starter fertilization.

4 | Discussion

This study was performed to investigate the effect of different starter fertilizers on current maize hybrids in Germany. The application of starter fertilizer is common practice in maize cultivation, but whether there are genotypes for which it can be omitted has not been thoroughly investigated yet. Now, however, that the application of fertilizers in agriculture, including the associated negative environmental effects, has come under focus and is becoming increasingly restricted, this question has gained social and political relevance and warrants scientific answers. We therefore screened the top 20 market leaders of German maize hybrid varieties in five locations under DAP (N+P), TSP (P), CAN (N) and Co (no) starter fertilization to assess potential P fertilizer reductions, evaluate the underlying genotypic components, identify superior genotypes regarding P supply, and draw conclusions for practical maize breeding.

4.1 | P as starter fertilizer and its interaction with other plant nutrients

The importance of a balanced nutrient management and specifically the role of nitrogen in fertilization is well known [5]. The results obtained in our study suggest a synergetic effect of a co-starter-fertilization of ammonium and phosphate. This combination had the strongest effect on early plant height measurements and thus youth development, and led to yield increases compared to the control treatment (Figs 1 and 3). Even though yield clearly represents the most important trait for the farmer, a successful youth development in farmers' fields is not to be underestimated. Interestingly, DAP starter fertilization also resulted in a slight increase of the P content, thus the amount of P that was successfully taken up by the plants and that is eventually removed from the field. This effect was extensively observed in former studies [25] and can be explained by the acidification ammonium causes in the soil, which enhances P uptake [25, 41]. More precisely, local ammonium supply stimulates the extension of the root system [42], which is caused by the accumulation of the plant hormone auxin [43]. In general, the soil conditions e.g. pH, anion and metal concentrations [21] as well as the effect of the previous crop and the crop rotation strongly impact the bioavailability of P in the rhizosphere [44]. In which combination P is given to the maize plants seems to be crucial for its successful conversion in the plant. The CAN treatment does not appear to unleash the available P in the soil, which is likely due to its lower acidifying potential compared to DAP [45].

Another interesting aspect when talking about the relationship of P with other plant nutrients is the consistent positive relationship of phosphorus with manganese, magnesium, potassium, sulfur, and zinc observed across all starter fertilizers (S4 Fig), which prevails also when looked at each starter fertilizer separately. Previous studies confirmed that potassium, manganese and magnesium were highly positively correlated with P in maize grains [46]. This underlines the need to check for example for a sufficiently high magnesium status of the fields, which—in case it is limited—should be applied as an efficient fertilizer combination as customary in trade. Taken together, these observations highlight the importance to keep in mind other nutrients besides P that promote maize youth development. When working on the improvement of phosphate-use-efficiency, we also have to consider the overall nutritional status in the soils, also with regard to suitable co-fertilization strategies and even planning of crop rotations, which determine the whole cropping system.

4.2 | Potential for optimizing the P balance on well-supplied soils

It is paramount to emphasize that all trial locations showed no P deficiency of the soils. By contrast, all soils can be classified as rich to very rich soils with regard to P availability (Table 1). For all further considerations, we therefore have to keep in mind that the starter fertilizer treatments took place on fields with an overall very good nutrient availability. While some parts of the world are challenged with P-deficient soils, in Germany this situation of well-supplied soils is rather the rule than the exception [18, 47]. Different studies underlined that current P stocks in the soils in Europe allow for sufficient P supply of the crops for several years with zero fertilization [18, 48]. In practical farming, the application of starter fertilizers is often simply conditioned by the availability of the corresponding sowing technique. Our results showed that only the combination of ammonium and phosphate (DAP) as starter fertilization resulted in significantly higher grain yields by on average +0.67 t/ha at the core location Hohenheim (Figs 1b and 3), as well as on average +0.4 t/ha over multiple locations, which corresponds to an increase of +5.34% and +3.6%, respectively (S6 Table). By contrast, the yield increase using only phosphate (TSP) as starter fertilizer only amounted to +1.2% across multiple locations. Thus, the commonly applied combination of ammonium and phosphate as starter fertilizer does have a positive effect on maize yield, at least on average across all 20 hybrid varieties.

Notably, however, performance without starter fertilizer has not been a breeding goal to date. Hence, there is a certain potential to reduce or omit P starter fertilizers and thereby gain leeway in the farm nutrient balance, even if this may come at the price of potential minor reductions in grain yield. At the same time, P surpluses on a farm but also on a regional level are or will be in the future restricted and fined by law, which makes it worthwhile for the farmer to thoroughly weigh additional fertilizer versus additional yield. We conclude that meaningful phosphate-use-efficiency in the context of well-saturated soils should be defined ideally as only minor yield reductions without extra P fertilization given as starter fertilizer.

4.3 | How to breed for phosphate-use-efficiency?

Two aspects determine if breeding of maize hybrids with a reduced need for starter fertilizer is possible and how it can be pursued. First, we need genetic variation regarding the response to reduced or no starter fertilizer, so that lines with no or only a minimal reduction in growth and yield can be selected. If so, the genotype-by-starter fertilizer interaction will determine under which conditions selection should be performed.

Our results show that there is variation regarding the response to starter fertilizer and thus the potential to omit it. Identifying and selecting genotypes that are high-yielding and maintain above-average performance regardless of the starter fertilization, is thus possible and can be considered a meaningful goal for breeding in Germany. As described in the literature [49], we also observed a shift of flowering dates due to the different starter fertilizers (S2 Fig). Generally speaking, the better the soil is supplied with P, the earlier the flowering takes place. In our case, however, this shift only amounted to less than one day and is of no practical relevance.

We observed neither a significant genotype-by-starter fertilizer interaction for the trait grain yield nor for P content (Fig 3). This suggests that breeders can select P-efficient lines independent of the soil P-status since generally the best genotypes perform the best no matter with or without starter fertilization. Nonetheless, further research is required to investigate whether this also holds true for soils with lower P availability than investigated in this study. With the expected restrictions for P fertilizer inputs ahead, breeders should still target to select under no P starter fertilizers conditions for obtaining better adapted material with regard to phosphate-use-efficiency.

For breeding purposes, more genotypes should be screened in more locations, including poorer P availability classes, and more importantly, the trials should be carried out in more years. It is known from other studies that the effect of starter fertilizers is extremely dependent on the environment [24, 50] and on the year [9]. As pointed out, the early phase of the field season in 2019 was extraordinary wet and cold (S1 Fig). The application of starter fertilizer may buffer against such adverse events and thus provide a kind of insurance for the farmer. This potential positive effect must be weighed against legal regulations restricting fertilizer use per farm. Obviously, the availability of varieties that do not require this external buffer in the form of starter fertilizer, but have a strong youth development and can cope with a certain level of abiotic stress genetically, would be an important means to reduce P input in our agricultural systems. More and more seed treatments that enhance the mobilization of P in the soils are currently entering the market and show additional ways of how a sustainable optimized P balance can be achieved in the future.

5 | Conclusions

Our study revealed that starter fertilizer treatments have a rather limited effect on grain yield but mainly show a positive effect on the youth development of maize. Breeding for phosphate-use-efficiency in the context of well-supplied soils, as present for example in Germany, should focus on genotypes that maintain high absolute grain yields even with a reduction of P inputs to zero. Selection of such phosphate-use-efficient varieties appears possible without taking the P level of the soil into account, since no substantial genotype-by-starter fertilizer interaction is expected under the P-rich soil conditions to be mostly found in Germany. In order to fulfill the clear social and political will of reducing fertilizers, plant breeding should contribute its part and provide varieties that allow the desired reduction of fertilizers without major financial disadvantages for the farmers.

Supporting information

S1 Table. Detailed description of hybrid varieties. Information of all 20 hybrids investigated in the field season 2019, including the breeding company, maturity (FAO groups go from early 170–220 to late 300–350), the main utilization ('B' denoting biogas, 'CCM' corn-cob-mix, 'G' grain and 'S' silage), and the companies standard seed treatment. The year of registration is given according to the federal plant variety office.
(PDF)

S2 Table. Starter fertilizer-location combinations. Control (Co), triple superphosphate (TSP), calcium ammonium nitrate (CAN), diammonium phosphate (DAP). In brackets the nitrogen and phosphorus content are given in percent.
(PDF)

S3 Table. Maize cultivation parameters. Given for the field season 2019 in each location.
(PDF)

S4 Table. Detailed description of trait assessments. Same methods were applied for all locations.
(PDF)

S5 Table. Repeatabilities in the single locations. Traits are abbreviated as follows: Plant height <55 days after sowing (DAS)(PH1), Plant height 56–60 DAS (PH2), Plant height 61–65 DAS (PH3), Plant height 66–70 DAS (PH4), Plant height 71–75 DAS (PH5), Plant height >75 DAS (PHfinal), ear height (EH), all measured in cm; days to anthesis (DTA) and days to silking (DTS), indicated in DAS; anthesis-silking-interval (ASI) in days; grain dry matter content (GDM) in percent; grain yield (GY) in tons dry matter/ha; Phosphorus grain concentration (P conc) measured with X-ray fluorescence in mg P/kg dry matter; and Phosphorus grain content (P cont) in kg P/ha. Control (Co, grey), starter fertilizers: triple superphosphate (TSP, purple), calcium ammonium nitrate (CAN, blue), diammonium phosphate (DAP, red).
(PDF)

S6 Table. Summary of the statistical analyses in the series. (i) Control (Co) vs. triple superphosphate (TSP) and (ii) Control (Co) vs. diammonium phosphate (DAP): Values are given for within each starter fertilizer treatment (indicated with Co, TSP, DAP, respectively) as well as across both starter fertilizer treatments. Minimum (Min), Mean, and Maximum (Max) is given based on the best linear unbiased estimators (BLUEs). σ^2_g denotes the genotypic variance, σ^2_l the location variance, σ^2_{gxt} the genotype-by-treatment-interaction variance, σ^2_{gxtxl} the genotype-by-treatment-by-location-interaction variance, σ^2_e, the error variance, and H^2 the broad-sense heritability. Traits are abbreviated as follows: plant height at BBCH stage ~ V4 (PH early), plant height at BBCH stage > R1 (PH late), ear height (EH), days to silking (DTS) given in in days after sowing (DAS), grain dry matter (GDM), grain yield (GY), phosphorus grain concentration (P grain conc), and phosphorus grain content (P cont). Significance levels

are shown as '*' (p-value < 0.05), '**' (p-value < 0.01), '***' (p-value < 0.001). All values are based on three locations (Co-TSP: Hohenheim, Eckartsweier, Dettingen; Co-DAP: Hohenheim, Einbeck, Saerbeck), except for P grain conc and P cont in the DAP series.
(PDF)

S7 Table. Raw data of hybrid trial.
(XLSX)

S1 Fig. Climograph and soil temperatures at the location Hohenheim. Daily precipitation rates [mm/d] and mean temperatures [˚C] of the year 2019. The dates of plant height measurements during the field season are indicated with dark green arrows, the period of the field season with a light green arrow. Soil temperatures at 2 cm, 20 cm, and 200 cm are shown in brown colors in the plot below.
(PDF)

S2 Fig. Histograms of specific traits. The traits final plant height (PHfinal), ear height (EH), days to anthesis (DTA), days to silking (DTS), and P concentration (Pconc) are depicted. Different letters indicate significant (p-value < 0.05) differences between starter fertilizers means. Starter fertilizers are abbreviated as Control (Co), triple superphosphate (TSP), calcium ammonium nitrate (CAN), diammonium phosphate (DAP).
(PDF)

S3 Fig. Correlation matrices of all investigated traits. Separated by starter fertilizers: control (Co), triple superphosphate (TSP), calcium ammonium nitrate (CAN), diammonium phosphate (DAP). Anthesis-silking-interval (ASI [d]), days to anthesis (DTA [d]), days to silking (DTS [d]), ear height (EH [cm]), grain dry matter content (GDM [%]), grain yield (GY [t/ha]), P grain concentration (Pconc [mg/kg]), P grain content (Pcont [kg/ha]), and plant heights (PH1, PH2, PH3, PHfinal [cm]). Red indicates negative correlations between traits, green positive correlations. Significance levels are shown as '.' (p-value < 0.1), '*' (p-value < 0.05), '**' (p-value < 0.01), '***' (p-value < 0.001).
(PDF)

S4 Fig. Network plot among 16 chemical elements. 120 grain samples of the core location HOH were analyzed, independent of starter fertilizer treatments. Positive Pearson correlations (r) are indicated in green, negative Pearson correlations in red.
(PDF)

S5 Fig. Heatmaps of all 20 maize hybrids and the investigated traits. Separated by starter fertilizers (control (Co), triple superphosphate (TSP), calcium ammonium nitrate (CAN), diammonium phosphate (DAP)): anthesis-silking-interval (ASI [d]), days to anthesis (DTA [d]), days to silking (DTS [d]), ear height (EH [cm]), grain dry matter (GDM [%]), grain yield (GY [t/ha]), P concentration (Pconc [mg/kg]), P content (Pcont [kg/ha]), and plant heights (PH1, PH2, PH3, PHfinal [cm]). Dark red indicates maximum, light yellow minimum trait values.
(PDF)

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References

1. Drobe M. Vorkommen und Produktion mineralischer Rohstoffe—ein Ländervergleich [Deposits and production of mineral raw materials—a country comparison] (2020). BGR—Bundesanstalt für Geowissenschaften und Rohstoffe [Federal Institute for Geosciences and Natural Resources]; December 2019.
2. Desmidt E, Ghyselbrecht K, Zhang Y, Pinoy L, van der Bruggen B, Verstraete W et al. Global Phosphorus Scarcity and Full-Scale P-Recovery Techniques: A Review. Critical Reviews in Environmental Science and Technology 2015; 45(4):336–84.
3. Cooper J, Lombardi R, Boardman D, Carliell-Marquet C. The future distribution and production of global phosphate rock reserves. Resources, Conservation and Recycling 2011; 57:78–86.
4. Niño-Savala AG, Zhuang Z, Ma X, Fangmeier A, Li H, Tang A et al. Cadmium pollution from phosphate fertilizers in arable soils and crops: an overview. Front. Agr. Sci. Eng 2019; 6(4):419.
5. Campbell NA, Reece JB, editors. Biologie. 8.th ed. Pearson Studium; 2009.
6. FAOSTAT. Data > Production > Crops; 2018. URL: http://www.fao.org/faostat/en/#data/QC.
7. Deutsches Maiskomitee e.V. Maisanbaufläche Deutschland in ha, 2018 (endgültig) und 2019 (endgültig) nach Bundesländern und Nutzungsrichtung in ha; 2020. URL: https://www.maiskomitee.de/web/upload/pdf/statistik/dateien_pdf/Maisanbauflaechen_D_je_BL_in_ha_2018-2019_endgueltig_20200206.pdf.
8. Teare ID, Wright DL. Corn Hybrid-Starter Fertilizer Interaction for Yield and Lodging. Crop Sci 1990; (30):1298–303.
9. Mascagni HJ Jr., Boquet DJ. Starter Fertilizer and Planting Date Effects on Corn Rotated with Cotton. J. Agron 1996; (88):975–82.
10. Federolf C-P, Westerschulte M, Olfs H-W, Trautz D. Optimizing Nitrogen and Phosphor Use Efficiencies from Liquid Manure by Slurry Injection to Reduce Environmental Pollution. Procedia Environmental Sciences 2015; 29:227–8.
11. FAOSTAT. Data > Inputs > Fertilizers by nutrient; 2017. URL: http://www.fao.org/faostat/en/#data/RFN.
12. Liu Y, Villalba G, Ayres RU, Schroder H. Global Phosphorus Flows and Environmental Impacts from a Consumption Perspective. J Ind Ecol 2008; 12(2):229–47.

13. Withers PJA, Forber KG, Lyon C, Rothwell S, Doody DG, Jarvie HP et al. Towards resolving the phosphorus chaos created by food systems. Ambio 2020; 49(5):1076–89. https://doi.org/10.1007/s13280-019-01255-1 PMID: 31542888

14. Macdonald GK, Jarvie HP, Withers PJA, Doody DG, Keeler BL, Haygarth PM et al. Guiding phosphorus stewardship for multiple ecosystem services. Ecosystem Health and Sustainability 2016; 2(12): e01251.

15. Fischer P, Pöthig R, Venohr M. The degree of phosphorus saturation of agricultural soils in Germany: Current and future risk of diffuse P loss and implications for soil P management in Europe. Sci Total Environ 2017; 599–600:1130–9. https://doi.org/10.1016/j.scitotenv.2017.03.143 PMID: 28511358

16. European Commission. Farm to Fork Strategy: For a fair, healthy and environmentally-friendly food system [#EUGreenDeal]; 2020. URL: https://ec.europa.eu/food/sites/food/files/safety/docs/f2f_action-plan_2020_strategy-info_en.pdf.

17. Verordnung über die Anwendung von Düngemitteln, Bodenhilfsstoffen, Kultursubstraten und Pflanzenhilfsmitteln nach den Grundsätzen der guten fachlichen Praxis beim Düngen [Ordinance on the Application of Fertilizers, Soil Additives, Cultivation Substrates and Plant Auxiliaries in Accordance with the Principles of Good Fertilizing Practice] (Düngeverordnung—DüV): DüV; 2017. URL: http://www.gesetze-im-internet.de/d_v_2017/D%C3%BCV.pdf.

18. Zhang J, Beusen AHW, van Apeldoorn DF, Mogollón JM, Yu C, Bouwman AF. Spatiotemporal dynamics of soil phosphorus and crop uptake in global cropland during the 20th century. Biogeosciences 2017; 14(8):2055–68.

19. Theobald TFH, Schipper M, Kern J. Phosphorus flows in Berlin-Brandenburg, a regional flow analysis. Resources, Conservation and Recycling 2016; 112:1–14.

20. Rothwell SA, Doody DG, Johnston C, Forber KJ, Cencic O, Rechberger H et al. Phosphorus stocks and flows in an intensive livestock dominated food system. Resources, Conservation and Recycling 2020; 163:105065.

21. Hinsinger P. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant Soil 2001; (237):173–95.

22. Wissuwa M, Ae N. Genotypic variation for tolerance to phosphorus defciency in rice and the potential for its exploitation in rice improvement. Plant Soil 2001; (237):275–86.

23. Leiser WL, Rattunde HFW, Piepho H-P, Weltzien E, Diallo A, Toure A et al. Phosphorous Efficiency and Tolerance Traits for Selection of Sorghum for Performance in Phosphorous-Limited Environments. Crop Sci 2015; 55(3):1152–62.

24. Torres LG, Rodrigues MC, Lima NL, Trindade TFH, Silva FFE, Azevedo CF et al. Multi-trait multi-environment Bayesian model reveals G × E interaction for nitrogen use efficiency components in tropical maize. PLoS One 2018; 13(6):e0199492. https://doi.org/10.1371/journal.pone.0199492 PMID: 29949626

25. Nkebiwe PM, Weinmann M, Bar-Tal A, Müller T. Fertilizer placement to improve crop nutrient acquisition and yield: A review and meta-analysis. Field Crops Res 2016; 196:389–401.

26. CycdesigN. URL: https://www.vsni.co.uk.

27. Bauer H-S. Meteorologischer Monatsbericht [Monthly Meteorological Report]. Universität Hohenheim; 2019 142.

28. Thun R, Hoffmann G, Herrmann R, Knickmann E. Die Untersuchung von Böden [The examination of soils]: neubearb. von Georg Hoffmann. 4th ed. Darmstadt; 2012. (Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik; vol 1).

29. Wiesler F. Mitteilungen: Neue Empfehlungen des VDLUFA für die P-Düngung [Announcements: New VDLUFA recommendations for P fertilization]; 2018. URL: https://www.vdlufa.de/Dokumente/WirUeberUns/Mitteilungen/Mitteilungen_02_18.pdf.

30. Bernal-Vasquez A-M, Utz H-F, Piepho H-P. Outlier detection methods for generalized lattices: a case study on the transition from ANOVA to REML. Theor Appl Genet 2016; 129(4):787–804. https://doi.org/10.1007/s00122-016-2666-6 PMID: 26883044

31. Cullis BR, Smith AB, Coombes NE. On the design of early generation variety trials with correlated data. JABES 2006; 11(4):381–93.

32. Piepho H-P, Möhring J. Computing heritability and selection response from unbalanced plant breeding trials. Genetics 2007; 177(3):1881–8. https://doi.org/10.1534/genetics.107.074229 PMID: 18039886

33. RStudio: Integrated Development Environment for R. Version 3.5.3. RStudio, PBC, Boston, MA URL; 2020. URL: http://www.rstudio.com/.

34. Butler DG, Cullis BR, Gilmour AR, Gogel BJ. ASReml-R reference manual: ASReml estimates variance components under a general linear mixed model by residual maximum likelihood (REML). Department of Primary Industries and Fisheries; 2009.

35. asremlPlus: Augments 'ASReml-R' in Fitting Mixed Models and Packages Generally in Exploring Prediction Differences. Version R package version 4.1–36; 2020. URL: https://CRAN.R-project.org/package=asremlPlus.

36. agricolae: Statistical Procedures for Agricultural Research. Version R package version 1.3–2; 2020. URL: https://CRAN.R-project.org/package=agricolae.

37. Pollard KS, Dudoit S, van der Laan MJ. Multiple Testing Procedures: R multtest Package and Applications to Genomics, in Bioinformatics and Computational Biology Solutions Using R and Bioconductor. Statistics for Biology and Health Series 2005:251–72.

38. ggpubr: 'ggplot2' Based Publication Ready Plots. Version R package version 0.2.5; 2020. URL: https://CRAN.R-project.org/package=ggpubr.

39. gplots: Various R Programming Tools for Plotting Data. Version R package version 3.0.3; 2020. URL: https://CRAN.R-project.org/package=gplots.

40. Epskamp S, Cramer AOJ, Waldorp LJ, Schmittmann VD, Borsboom D. qgraph: Network Visualizations of Relationships in Psychometric Data. Journal of Statistical Software 2012; 48(4):1–18. Available from: URL: http://www.jstatsoft.org/v48/i04/.

41. Pedersen IF, Sørensen P, Rasmussen J, Withers PJA, Rubaek GH. Fertilizer ammonium: nitrate ratios determine phosphorus uptake by young maize plants. J. Soil Sci. Plant Nutr 2019; 182(4):541–51.

42. Jing J, Zhang F, Rengel Z, Shen J. Localized fertilization with P plus N elicits an ammonium-dependent enhancement of maize root growth and nutrient uptake. Field Crops Res 2012; 133:176–85.

43. Meier M, Liu Y, Lay-Pruitt KS, Takahashi H, von Wirén N. Auxin-mediated root branching is determined by the form of available nitrogen. Nat Plants 2020; 6(9):1136–45. https://doi.org/10.1038/s41477-020-00756-2 PMID: 32917974

44. Hallama M, Pekrun C, Lambers H, Kandeler E. Hidden miners—the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. Plant Soil 2019; 434(1–2):7–45.

45. Grunert M. Nährstoffgehalte ausgewählter mineralischer N-, P- und K-Dünger. Freistaat Sachsen; 03/2019. Datensammlung Düngerecht.

46. Lim S, Yi G. Investigating seed mineral composition in Korean landrace maize (Zea mays L.) and its kernel texture specificity. J. Integr. Agric 2019; 18(9):1996–2005.

47. Merbach W, Herbst F, Eißner H, Schmidt L, Deubel A. Influence of different long-term mineral–organic fertilization on yield, nutrient balance and soil C and N contents of a sandy loess (Haplic Phaeozem) in middle Germany. Archives of Agronomy and Soil Science 2013; 59(8):1059–71.

48. Nawara S, van Dael T, de Cooman E, Elsen A, Merckx R, Smolders E et al. Testing soil phosphorus in a depleting P scenario: an accelerated soil mining experiment. European Journal of Soil Science 2018; 69(5):804–15.

49. Xu C, Zhang H, Sun J, Guo Z, Zou C, Li W-X et al. Genome-wide association study dissects yield components associated with low-phosphorus stress tolerance in maize. Theor Appl Genet 2018; 131(8):1699–714. https://doi.org/10.1007/s00122-018-3108-4 PMID: 29754325

50. Ewing PM, Runck BC, Kono TYJ, Kantar MB. The home field advantage of modern plant breeding. PLoS One 2019; 14(12):e0227079. https://doi.org/10.1371/journal.pone.0227079 PMID: 31877180

Optimizing the P balance: How do modern maize hybrids react to different starter fertilizers?

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Supporting information

TABLE S1. Detailed description of hybrid varieties. Information of all 20 hybrids investigated in the field season 2019, including the breeding company, maturity (FAO groups go from early 170-220 to late 300-350), the main utilization ('B' denoting biogas, 'CCM' corn-cob-mix, 'G' grain and 'S' silage), and the companies standard seed treatment. The year of registration is given according to the federal plant variety office.

Variety	**Company**	**Year of registration**	**Maturity**	**Main utilization**	**Treatment**
AGROPOLIS	Agro Mais	2015	S240	S/B/CCM	Mesurol + TMTD
AMAROC	Agro Mais	2016	S230	S/B/CCM	Mesurol + TMTD
AMAVERITAS	Agro Mais	2017	S240/K240	S/G/B	Mesurol + TMTD
BENEDICTIO KWS	KWS	2016	S230/K230	S/G	Mesurol + TMTD
BERNARDINIO KWS	KWS	2018	S240/K240	S/G	Mesurol + TMTD
ES METRONOM	Euralis	2014	S240/K240	S/G/B	Mesurol + Maxim XL
GEOXX	RAGT	2010	S240/K240	S/G/B	Mesurol + Maxim XL + Biofortifier (Geoxx Gold)
HULK	agaSAAT GmbH	2013	S250-260/K250-260	S/G/B/CCM	Maisprotektor (Fungizid + Vogelfraß)
KWS FIGARO	KWS	2016	S250/K250	S/G	Mesurol + TMTD
KWS STABIL	KWS	2013	S200/K200	S/G	Mesurol + TMTD
LG 30.222	Limagrain	2010	S210/K220	S/G	Mesurol + Maxim XL
LG 30.258	Limagrain	2016	S240/K240	S/G/B	Mesurol + Maxim XL
LG 32.16	Limagrain	2007	S260/K240	S/G/B	Mesurol + Maxim XL
P 8666	Pioneer	2017	S260/K250	S/G/B	Mesurol + Maxim XL
P 8329	Pioneer	2016	S250/K240	G	Mesurol + Maxim XL
RICARDINIO	KWS	2008	S230/K220	S/G	Mesurol + TMTD
SIMPATICO KWS	KWS	2014	S250/K260	S/G	Mesurol + TMTD
SY KARDONA	Syngenta	2014	S250/K240	S/G	Mesurol + Maxim XL
SY TALISMAN	Syngenta	2015	S220/K230	S/G	Mesurol + Maxim XL
WALTERINIO KWS	KWS	2015	S270/K270	S/G	Mesurol + TMTD

TABLE S2. **Starter fertilizer-location combinations.** Control (Co), triple superphosphate (TSP), calcium ammonium nitrate (CAN), diammonium phosphate (DAP). In brackets the nitrogen and phosphorus content are given in percent.

Starter fertilizers (N / P)[%]	**Co (- / -)**	**TSP (- / 20))**	**CAN (26 / -)**	**DAP (18 / 20)**
Hohenheim	0 kg/ha	115 kg/ha	77 kg/ha	115 kg/ha
Eckartsweier	0 kg/ha	115 kg/ha	-	-
Dettingen	0 kg/ha	115 kg/ha	-	-
Einbeck	0 kg/ha	-	-	100 kg/ha
Saerbeck	0 kg/ha	-	-	116 kg/ha

TABLE S3. Maize cultivation parameters. Given for the field season 2019 in each location.

Parameters [Unit]	**Sowing** [Date]	**Sowing Depth** [cm]	**Sowing Density** [plants/m^2]	**Plot Size** [m^2]	**Harvest** [Date]
Hohenheim	April 29th	6	8.8	7.5	October 15th&16th
Eckartsweier	April 23th	4.5	8.8	7.5	September 24th
Dettingen	May 6th	5	8.8	7.5	October 29th
Einbeck	May 1st	5.5	10	18	October 17th
Saerbeck	April 27th	5	10	15.6	October 10th

TABLE S4. **Detailed description of trait assessments.** Same methods were applied for all locations.

Trait	Abbreviation	Description	Unit [ ]
Plant height	PH	The average height of three representative individuals from the ground to the straigthened youngest leave (V-stages) and up to tassel tip for the final measurement (R-stage), respectively	cm
Ear height	EH	The average height of three representative individuals from the ground to the internode of the top (uppermost) ear	cm
Days to anthesis	DTA	Male flowering: 50% pollen shedding in one plot	days after sowing
Days to silking	DTS	Female flowering: 50% silks visible in one plot	days after sowing
Anthesis-silking-interval	ASI	Days between male and female flowering	days
Grain dry matter content	GDM	Percentage of dry matter after 72h at 110°C in relation to fresh weight at harvest	%
Grain yield	GY	Weight of all threshed grain per plot, corrected for its water content, recalculated for one hectare	t dry matter/ha
Phosphorus grain concentration	Pconc	The average concentration of phosphorus in the maize kernels, measured by means of X-ray fluorescence; calibration obtained by inductively coupled plasma optical emission spectrometry (ICP-OES)	ppm = mg/kg
Phosphorus grain content	Pcont	Phosphorus concentration * grain yield	kg/ha

TABLE S5. Repeatabilities in the single locations. Traits are abbreviated as follows: Plant height <55 days after sowing (DAS)(PH1), Plant height 56-60 DAS (PH2), Plant height 61-65 DAS (PH3), Plant height 66-70 DAS (PH4), Plant height 71-75 DAS (PH5), Plant height >75 DAS (PHfinal), ear height (EH), all measured in cm; days to anthesis (DTA) and days to silking (DTS), indicated in DAS; anthesis-silking-interval (ASI) in days; grain dry matter content (GDM) in percent; grain yield (GY) in tons dry matter/ha; Phosphorus grain concentration (P conc) measured with X-ray fluorescence in mg P/kg dry matter; and Phosphorus grain content (P cont) in kg P/ha. Control (Co, grey), starter fertilizers: triple superphosphate (TSP, purple), calcium ammonium nitrate (CAN, blue), diammonium phosphate (DAP, red).

	PH1 [cm]	PH2 [cm]	PH3 [cm]	PH4 [cm]	PH5 [cm]	PHfinal [cm]	EH [cm]	DTA [DAS]	DTS [DAS]	ASI [d]	GDM [%]	GY [t DM /ha]	P conc [mg/kg DM]	P cont [kg/ha]
(i)	**Hohenheim**													
Co	0.85	0.80	0.83			0.71	0.86	0.95	0.96	0.84	0.97	0.62	0.80	0.67
TSP	0.64	0.82	0.83			0.78	0.83	0.93	0.95	0.79	0.91	0.49	0.92	0.50
CAN	**0.86**	**0.75**	**0.85**			**0.79**	**0.91**	**0.94**	**0.97**	**0.85**	**0.97**	**0.40**	**0.86**	**0.36**
DAP	0.65	0.66	0.67			0.91	0.87	0.91	0.95	0.73	0.96	0.62	0.94	0.69
(ii)	**Eckartsweier**													
Co				0.83		0.67	0.82		0.91		0.94	0.41	0.93	0.40
TSP				0.67		0.84	0.81		0.93		0.96	0.66	0.94	0.79
(iii)	**Dettingen**													
Co	0.51				0.77	0.81	0.82				0.97	0.35	0.92	0.64
TSP	0.70				0.90	0.81	0.82				0.97	0.60	0.93	0.66
(iv)	**Einbeck**													
Co	0.72					0.83	0.92				0.96	0.66		
DAP	0.64					0.82	0.97				0.98	0.85		
(v)	**Saerbeck**													
Co		0.62						0.87	0.90	0.51	0.93	0.36		
DAP		0.65						0.92	0.95	0.54	0.98	0.55		

TABLE S6. **Summary of the statistical analyses in the series.** (i) Control (Co) vs. triple superphosphate (TSP) and (ii) Control (Co) vs. diammonium phosphate (DAP): Values are given for within each starter fertilizer treatment (indicated with Co, TSP, DAP, respectively) as well as across both starter fertilizer treatments. Minimum (Min), Mean, and Maximum (Max) is given based on the best linear unbiased estimators (BLUEs). σ^2_g denotes the genotypic variance, σ^2_l the location variance, σ^2_{gxt} the genotype-by-treatment-interaction variance, σ^2_{gxtxl} the genotype-by-treatment-by-location-interaction variance, σ^2_e, the error variance, and H^2 the broad-sense heritability. Traits are abbreviated as follows: plant height at BBCH stage ~ V4 (PH early), plant height at BBCH stage > R1 (PH late), ear height (EH), days to silking (DTS) given in in days after sowing (DAS), grain dry matter (GDM), grain yield (GY), phosphorus grain concentration (P grain conc), and phosphorus grain content (P cont). Significance levels are shown as '*' (p-value < 0.05), '**' (p-value < 0.01), '***' (p-value < 0.001). All values are based on three locations (Co-TSP: Hohenheim, Eckartsweier, Dettingen; Co-DAP: Hohenheim, Einbeck, Saerbeck), except for P grain conc and P cont in the DAP series.

	PH early [cm]	PH late [cm]	EH [cm]	DTS [DAS]	GDM [%]	GY [t DM/ha]	P grain conc [mg/kg DM]	P cont [kg P/ha]
(i) Co vs TSP								
Min (Co)	106.67	269.17	115.28	81.25	64.52	11.08	2132	25.13
Min (TSP)	108.67	266.94	110.28	80.25	64.73	10.91	2127	25.94
Mean (Co)	120.04	296.52	137.56	83.59	69.70	11.75	2362	27.74
Mean (TSP)	124.56	293.11	131.41	82.69	70.39	11.89	2379	28.34
Max (Co)	130.42	315.83	159.17	86.50	74.65	12.78	2742	30.69
Max (TSP)	133.75	310.83	151.17	86.25	74.93	12.99	2796	31.32
σ^2_g (Co)	40.84***	128.32***	154.99***	2.74***	4.82***	<0.01	21633.27***	535.25
σ^2_g (TSP)	47.84***	109.12***	114.18***	2.44***	5.52***	0.14	27897.23***	863.19
σ^2_{gxl} (Co)	<0.01	<0.01	<0.01	0.45**	1.13***	0.20*	6564.03**	2048.21**
σ^2_{gxl} (TSP)	<0.01	21.14	21.88	0.62***	1.17***	0.24*	6941.92***	2498.12*
σ^2_e (Co)	35.68	79.88	51.77	0.46	0.56	0.50	6876.71	3466.93
σ^2_e (TSP)	23.59	62.20	57.78	0.37	0.67	0.47	4730.04	3580.09
H^2 (Co)	0.87	0.91	0.94	0.92	0.91	0.02	0.87	0.30
H^2 (TSP)	0.92	0.86	0.89	0.90	0.92	0.47	0.90	0.38
σ^2_g	45.18***	118.06***	117.32***	2.57***	5.15***	0.08	24392.19***	708.67
σ^2_l	3.98**	862.02***	103.27***	6.34***	15.24***	0.59***	5674.53***	7303.70***
σ^2_{gxt}	<0.01	11.02	<0.01	0.01	<0.01	<0.01	333.71	<0.01
σ^2_{gxl}	2.01	11.02	10.86*	0.56***	1.22***	0.28***	6780.18***	2442.89***
σ^2_{gxtxl}	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
σ^2_e	26.54	60.86	48.07	0.39	0.56	0.41	5790.10	3337.99
H^2	0.90	0.90	0.91	0.91	0.91	0.34	0.88	0.34

(ii) Co vs DAP								
Min (Co)	119.83	285.00	110.00	82.75	63.08	10.50	2107$^{\$}$	25.69$^{\$}$
Min (DAP)	132.56	291.67	113.17	80.00	64.03	10.88	2175$^{\$}$	26.13$^{\$}$
Mean (Co)	134.93	311.46	135.31	86.19	67.28	11.16	2383$^{\$}$	29.66$^{\$}$
Mean (DAP)	146.46	313.90	135.10	83.84	68.12	11.56	2429$^{\$}$	30.74$^{\$}$
Max (Co)	144.22	336.67	162.92	89.25	72.51	12.00	2706$^{\$}$	35.71$^{\$}$
Max (DAP)	154.56	334.58	165.00	87.75	72.96	12.12	2836$^{\$}$	34.96$^{\$}$
σ^2_g (Co)	39.09***	140.34***	171.04***	2.85***	4.10***	0.09*	19842.73**$^{\$}$	2211.47*$^{\$}$
σ^2_g (DAP)	32.48***	112.83***	149.75***	4.49**	4.48***	0.02	25180.66***$^{\$}$	2559.33*$^{\$}$
σ^2_{gxl} (Co)	3.54	<0.01	14.07	0.43**	0.45***	0.09	n.a.	n.a.
σ^2_{gxl} (DAP)	8.09	10.49	29.70***	1.52***	0.40***	0.25**	n.a.	n.a.
σ^2_e (Co)	26.54	70.18	43.02	0.49	0.45	0.30	10000.86$^{\$}$	2980.52$^{\$}$
σ^2_e (DAP)	42.06	38.19	22.36	0.63	0.30	0.27	4152.71$^{\$}$	4903.24$^{\$}$
H^2 (Co)	0.87	0.92	0.94	0.93	0.95	0.70	0.80$^{\$}$	0.67$^{\$}$
H^2 (DAP)	0.77	0.92	0.92	0.88	0.96	0.09	0.92$^{\$}$	0.50$^{\$}$
σ^2_g	32.69***	125.21***	162.28***	3.71***	4.34***	0.07*	n.a.	n.a.
σ^2_l	310.30***	711.65***	49.25***	0.58*	0.97***	2.35***	n.a.	n.a.
σ^2_{gxt}	2.22	0.90	<0.01	<0.01	<0.01	<0.01	n.a.	n.a.
σ^2_{gxl}	8.50**	6.73	20.08***	0.60**	0.26***	0.09*	n.a.	n.a.
σ^2_{gxtxl}	<0.01	<0.01	<0.01	0.34**	0.12*	0.07	n.a.	n.a.
σ^2_e	32.31	53.10	32.89	0.56	0.38	0.28	n.a.	n.a.
H^2	0.78	0.92	0.93	0.93	0.97	0.46	n.a.	n.a.

$^{\$}$: only assessed for the location HOH

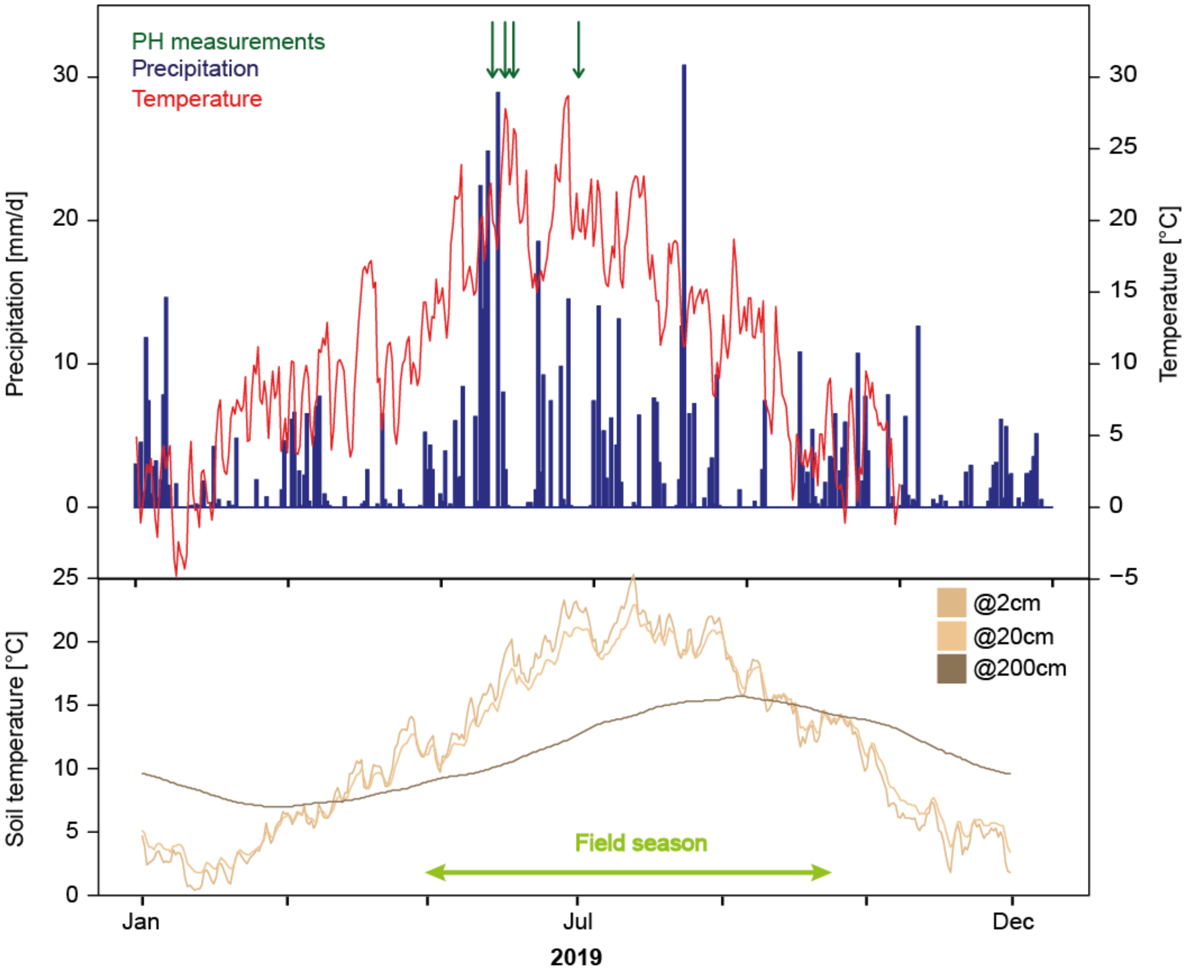

FIGURE S1. Climograph and soil temperatures at the location Hohenheim. Daily precipitation rates [mm/d] and mean temperatures [°C] of the year 2019. The dates of plant height measurements during the field season are indicated with dark green arrows, the period of the field season with a light green arrow. Soil temperatures at 2 cm, 20 cm, and 200 cm are shown in brown colors in the plot below.

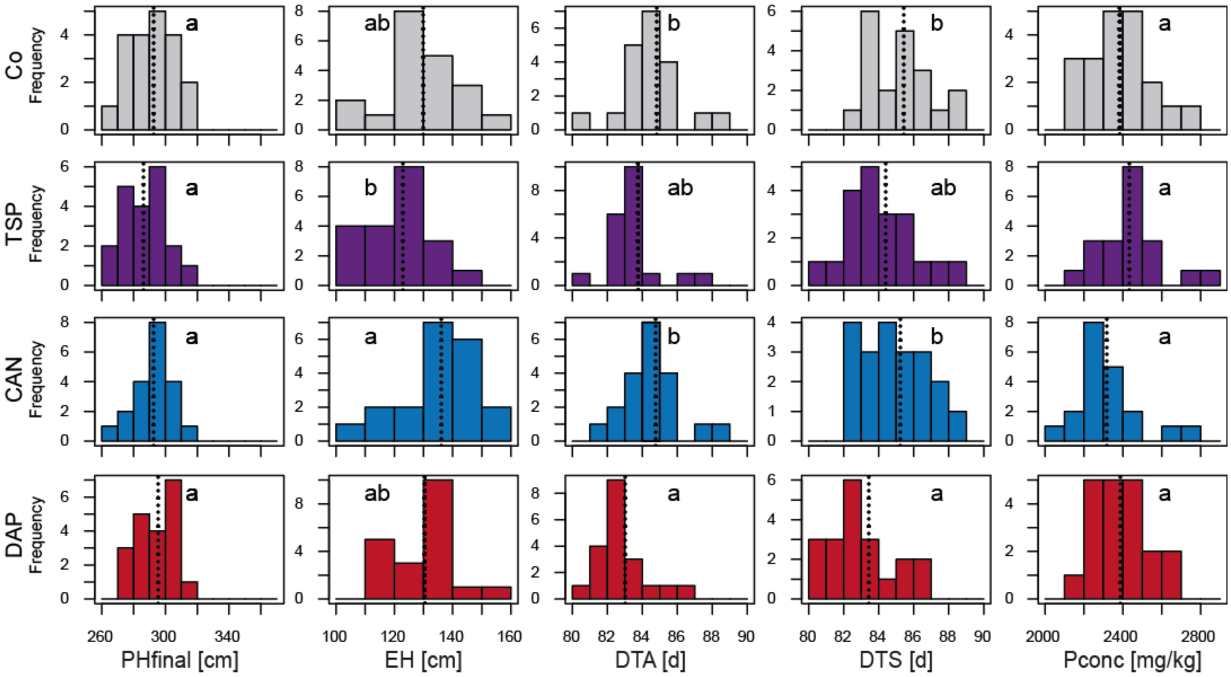

FIGURE S2. Histograms of specific traits. The traits final plant height (PHfinal), ear height (EH), days to anthesis (DTA), days to silking (DTS), and P concentration (Pconc) are depicted. Different letters indicate significant ($p\text{-value} < 0.05$) differences between starter fertilizers means. Starter fertilizers are abbreviated as Control (Co), triple superphosphate (TSP), calcium ammonium nitrate (CAN), diammonium phosphate (DAP).

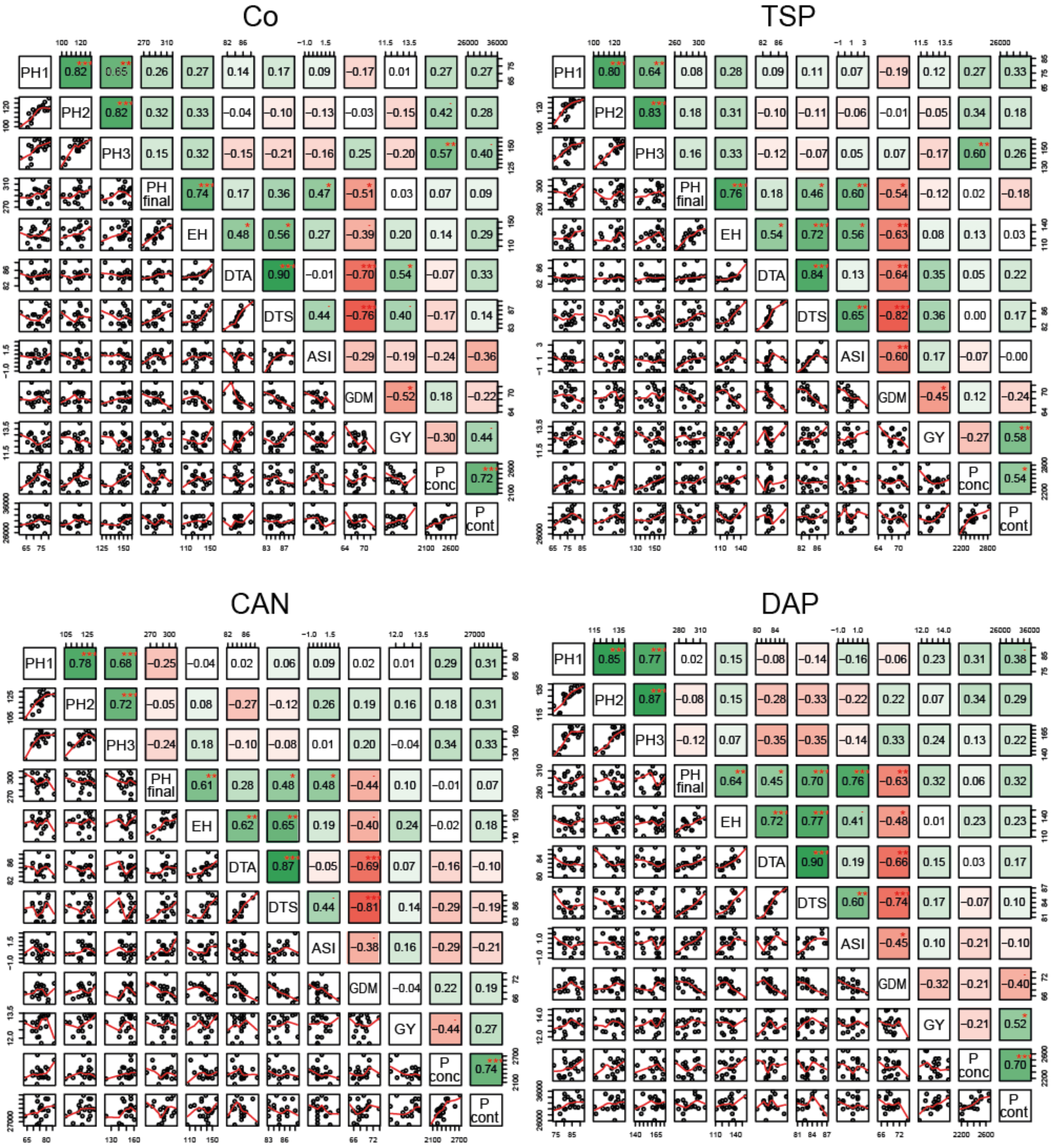

FIGURE S3. Correlation matrices of all investigated traits. Separated by starter fertilizers: control (Co), triple superphosphate (TSP), calcium ammonium nitrate (CAN), diammonium phosphate (DAP). Anthesis-silking-interval (ASI [d]), days to anthesis (DTA [d]), days to silking (DTS [d]), ear height (EH [cm]), grain dry matter content (GDM [%]), grain yield (GY [t/ha]), P grain concentration (Pconc [mg/kg]), P grain content (Pcont [kg/ha]), and plant heights (PH1, PH2, PH3, PHfinal [cm]). Red indicates negative correlations between traits, green positive correlations. Significance levels are shown as '.' (p-value < 0.1), '*' (p-value < 0.05), '**' (p-value < 0.01), '***' (p-value < 0.001).

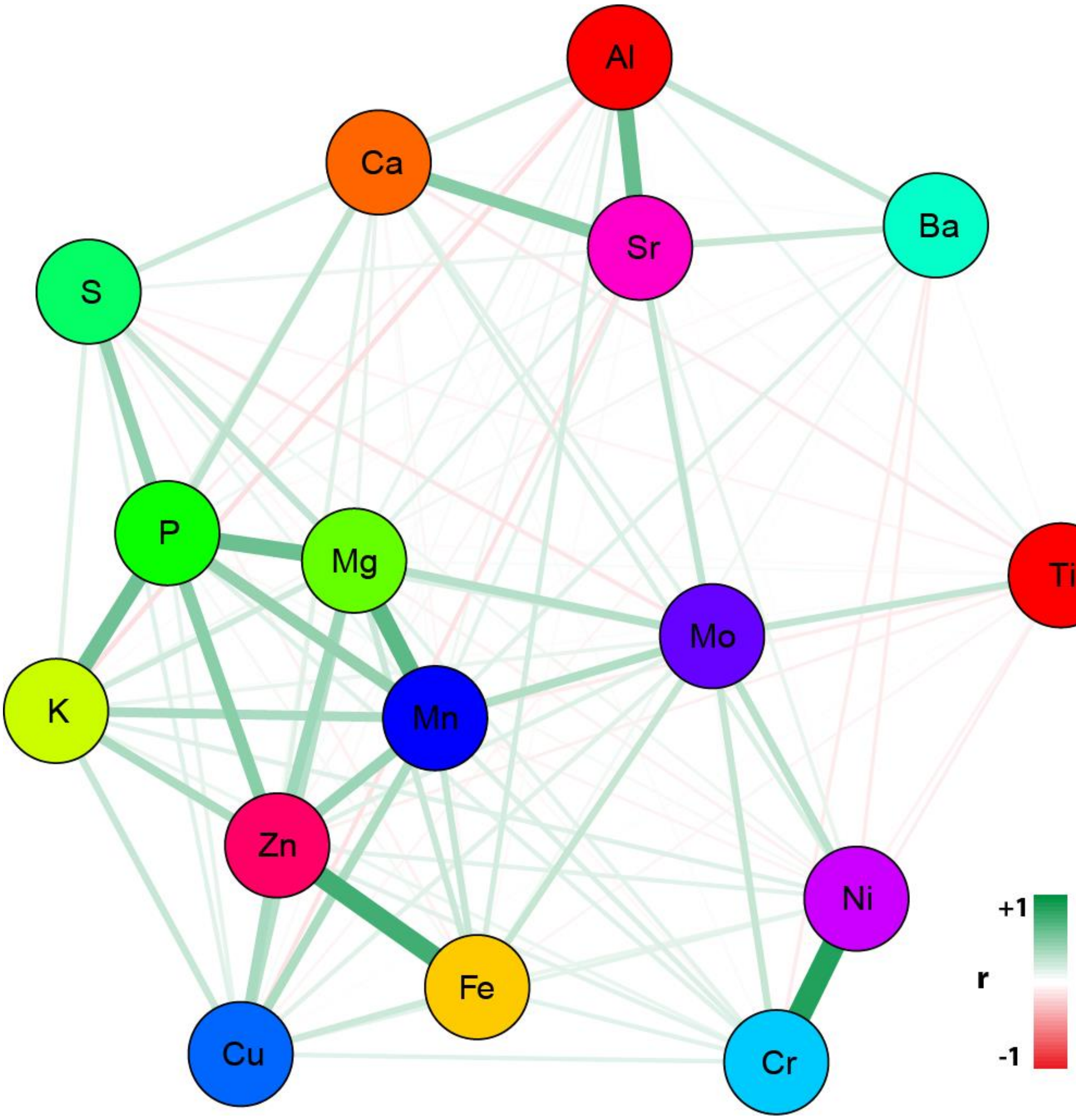

FIGURE S4. Network plot among 16 chemical elements. 120 grain samples of the core location HOH were analyzed, independent of starter fertilizer treatments. Positive Pearson correlations (r) are indicated in green, negative Pearson correlations in red.

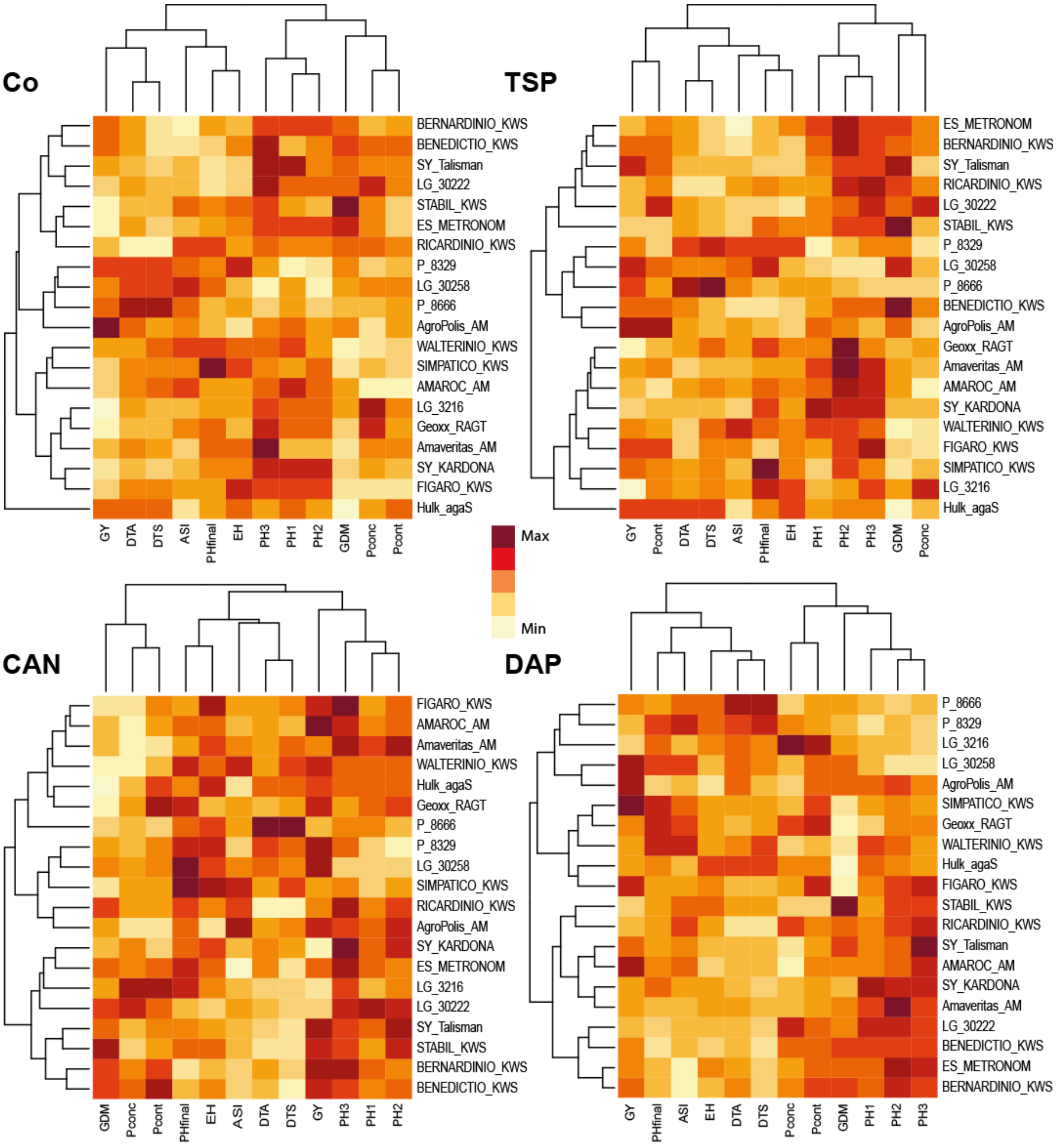

FIGURE S5. Heatmaps of all 20 maize hybrids and the investigated traits. Separated by starter fertilizers (control (Co), triple superphosphate (TSP), calcium ammonium nitrate (CAN), diammonium phosphate (DAP)): anthesis-silking-interval (ASI [d]), days to anthesis (DTA [d]), days to silking (DTS [d]), ear height (EH [cm]), grain dry matter (GDM [%]), grain yield (GY [t/ha]), P concentration (Pconc [mg/kg]), P content (Pcont [kg/ha]), and plant heights (PH1, PH2, PH3, PHfinal [cm]). Dark red indicates maximum, light yellow minimum trait values.

Unraveling the potential of phenomic selection within and among diverse breeding material of maize (*Zea mays* L.)

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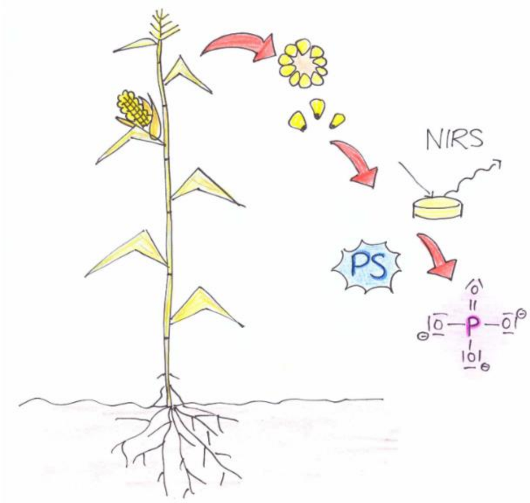

Figure 6 Graphical excerpt of publication 2. The central trait P concentration in the grain was - among others - predicted by the phenomic selection (PS) approach, which uses near-infrared spectra (NIRS) instead of marker data to predict traits.

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Investigation

Unraveling the potential of phenomic selection within and among diverse breeding material of maize (*Zea mays* L.)

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Abstract

Genomic selection is a well-investigated approach that facilitates and supports selection decisions for complex traits and has meanwhile become a standard tool in modern plant breeding. Phenomic selection has only recently been suggested and uses the same statistical procedures to predict the targeted traits but replaces marker data with near-infrared spectroscopy data. It may represent an attractive low-cost, high-throughput alternative but has not been sufficiently studied until now. Here, we used 400 genotypes of maize (*Zea mays* L.) comprising elite lines of the Flint and Dent heterotic pools as well as 6 Flint landraces, which were phenotyped in multienvironment trials for anthesis-silking-interval, early vigor, final plant height, grain dry matter content, grain yield, and phosphorus concentration in the maize kernels, to compare the predictive abilities of genomic as well as phenomic prediction under different scenarios. We found that both approaches generally achieved comparable predictive abilities within material groups. However, phenomic prediction was less affected by population structure and performed better than its genomic counterpart for predictions among diverse groups of breeding material. We therefore conclude that phenomic prediction is a promising tool for practical breeding, for instance when working with unknown and rather diverse germplasm. Moreover, it may make the highly monopolized sector of plant breeding more accessible also for low-tech institutions by combining well established, widely available, and cost-efficient spectral phenotyping with the statistical procedures elaborated for genomic prediction - while achieving similar or even better results than with marker data.

Keywords: phenomic selection; genomic selection; RR-BLUP; NIRS; predictive ability; landraces; maize breeding

Introduction

Plant breeding was revolutionized by the advent of genotypic information in the form of marker data. The seemingly obvious advantage was to introduce science to a subject formerly largely deemed as art (Jiang et al. 2020). This development has only occurred in the last decades with many approaches from quantitative trait loci (QTL) mapping (Würschum 2012) to genome editing developed since then (Benavente and Giménez 2021). Among these approaches is *genomic selection*, which was first implemented in animal breeding (Meuwissen et al. 2001), but has meanwhile also become a standard breeding tool for the prediction and subsequent selection of complex traits in plant breeding. Selection using genomic predictions was shown to lead to higher gains compared to pure phenotypic selection (Bernardo 2021b). There are different models that can be applied to perform genomic selection (Heffner et al. 2009) and many studies elaborating on, refining and comparing genomic selection approaches differing in their assumption with regard to the marker effect distribution are available (e.g. Resende et al. 2012; Thavamanikumar et al. 2015). Ridge regression best linear unbiased prediction (RR-BLUP), which assumes a homogeneous variance of all marker effects on the entire genome, has proven to be a robust method for predicting traits with many small-effect QTL (Heslot et al. 2012), as it generally results in high predictive abilities, expressed as the correlation between predicted and observed trait values. Besides QTL effects, the methods used for genomic prediction also exploit relatedness among individuals to achieve their predictive ability (Schopp et al. 2017; Bernardo 2021a). Special attention was also given to the role of the training set in genomic selection as its composition and relatedness to the prediction set are known to strongly impact prediction accuracies (e.g. Riedelsheimer et al. 2013; Schopp et al. 2015; Zhu et al. 2021). In general, genomic prediction is utilized to assist in a better use of available financial resources within the breeding process. It should be noted though, that generating marker data by genotyping is still cost-intensive and - if not outsourced - requires a certain laboratory infrastructure. There have been several advances and attempts in different crop species to include omics data other than genomics as predictors, but to date these are more difficult to obtain and more expensive than genotypic data

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(Riedelsheimer et al. 2012; Westhues et al. 2017; Schrag et al. 2018; Stich et al. 2020; Knoch et al. 2021).

Relatively new is the concept of *phenomic selection*, which was first proposed by Rincent et al. (2018) with the species poplar and wheat. The basic principle is to use near-infrared spectroscopy (NIRS) data instead of the marker data of each genotype for prediction. Hence, all wavelength information is used jointly. By this, the approach differs by definition from the traditional and ordinary NIRS application, which makes use of calibrations of only few wavelengths to predict specific traits (e.g. Melchinger et al. 1986). Several studies have successfully incorporated a set of wavelengths into the prediction models (Aguate et al. 2017; Hayes et al. 2017; Montesinos-López et al. 2017; Krause et al. 2019; Parmley et al. 2019; Galán et al. 2020); however, very few have investigated the innovative approach of phenomic selection as an alternative or complementary tool to facilitate selection decisions in breeding (Rincent et al. 2018; Lane et al. 2020). As a consequence, several questions with regard to the potential of applying phenomic selection in plant breeding still remain open. By providing answers to these questions, we might be able to broadly and routinely utilize this approach in breeding and thereby eliminate the need for genotyping (Lane et al. 2020). This might specifically empower less high-tech breeding companies and institutions, since an NIR spectrometer is rather easy to buy and maintain compared to a genotyping laboratory.

Hence, this study was motivated to contribute filling this gap, particularly regarding the performance of genomic and phenomic prediction within and among diverse groups of breeding material. To this end, we employed a set of 400 genotypes of maize - half elite material from two heterotic groups, half from 6 diverse landraces - to predict 6 traits relevant for maize breeding, i.e. anthesis-silking-interval, early vigor, final plant height, grain dry mattercontent, grain yield, and phosphorus concentration in the maize kernels. We particularly aimed to (1) estimate the predictive abilities of phenomic and genomic prediction within groups and among groups and to compare both approaches, (2) assess how population structure may influence predictive abilities of both approaches, (3) evaluate the impact of training set composition on the predictive abilities obtained by phenomic and genomic prediction, and (4) draw conclusions for practical plant breeding.

Materials and methods

For a better overview, the different steps of data processing are visualized in Supplementary Fig. 1. Overall, 3 threads were defined, namely phenotypic, genotypic, and NIRS data processing.

Phenotypic data

Field experiments

In total, 400 genotypes were investigated in this study. These comprised 100 elite Dent (ED) lines, 100 elite Flint (EF) lines and 200 lines from 6 Flint landraces (LR) (Fig. 1a). The genotypes of the landraces group were immortalized as doubled-haploid lines and are comprised of the following 6 landraces: Campan-Galade (CG; $n = 11$) originating from France, Gelber Badischer (GB; $n = 33$) from Germany, Sankt Galler Rheintaler (RT; $n = 14$) from Switzerland, Satu Mare (SM; $n = 53$) from Romania, Strenzfelder (SF; $n = 30$) from Germany, and Walliser (WA; $n = 59$) from Switzerland. The plant material has been described in previous studies (Böhm et al. 2017; Würschum et al. 2021). Three different location-year-combinations served as environments for the phenotypic data used in this study, the experimental station for plant breeding in Hohenheim (HOH; 48°43′05.7″N, 9°11′20.8″E; 389 m above sea level) during the field seasons 2019 and 2020 and the experimental station Eckartsweier (EWE; 48°32′24.7″N, 7°51′15.1″E; 139 m above sea level) during the field season 2020. Average precipitation and mean temperatures over the last 5 years of the two locations amounted to 663.3 mm, 10.3°C and 683.3 mm, 11.5°C, respectively (Agrometeorology Baden-Württemberg 2021). The field trials were laid out as alpha lattices, designed with the software CycdesigN (VSN International 2018), and each genotype was replicated twice. Standard seed and field treatments were applied before and during the field season. The net plot size was 6 m^2 and the sowing density was 8.66 plants/m^2. The following 6 phenotypic traits were assessed in this study in all location-year-combinations corresponding to 2,400 plots in total: anthesis-silking interval (ASI; days between 50% pollen shedding and 50% visible silks of a plot), early vigor (EV; 1 = "very poor" to 9 = "very vigorous" score), final plant height (Final PH; for HOH measured as the mean of 3 single plants, for EWE as one estimated value over the whole plot, given in cm), grain dry matter content (in %), grain yield (GYield in t/ha), and P concentration in the maize kernels [Pconc; for HOH_2019 and EWE_2020; milled to 1 mm, measured by means of X-ray fluorescence (Bruker, Billerica, MA, USA) in ppm].

Best linear unbiased estimation

The raw data were subjected to the Bonferroni-Holm outlier detection (Bernal-Vasquez et al. 2016) using the R-package "multtest" (Pollard et al. 2005). The hereafter described mixed model was applied for the multiple environment analyses using the software ASRemL-R 3.0 (Butler et al. 2009):

$$y_{ijkl} = \mu + g_i + e_j + (ge)_{ij} + r_{jk} + b_{jkl} + \varepsilon_{ijkl} \qquad (1)$$

where the phenotypic trait value y_{ijkl} is explained by the overall mean μ, the factor genotype g_i, the location-year-combinations denoted here as environments e_j, the interaction of genotype and environment $(ge)_{ij}$, the design variables replication r_{jk} and block effect b_{jkl} each nested within the environment, and the error term ε_{ijkl}, for which a homogeneous variance was assumed.

By taking all factors as random into the model, broad-sense heritabilities (Cullis et al. 2006; Piepho and Möhring 2007) of the traits were calculated. For the case of calculating the group-specific variance components, we introduced dummy variables and derived broad-sense heritabilities for each group by the formula (Hallauer et al. 2010):

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{g\times e}^2}{n_e} + \frac{\sigma_\varepsilon^2}{n_e * n_r}} \qquad (2)$$

where σ_g^2 denotes the genotypic variance in the group, $\sigma_{g\times e}^2$ denotes the group-specific genotype-by-environment interaction variance, σ_ε^2 denotes the variance of the error, and n_e and n_r denote the number of environments and replications, respectively. Using the factor genotype as fixed in the mixed model of Equation (1), we then calculated best linear unbiased estimates (BLUEs). These BLUEs were subsequently used as phenotypic trait values for all further analyses. The R-package "agricolae" was used to perform significance tests ($\alpha = 0.05$) of the group means (de Mendiburu 2020). The phenotypic raw data as well as the hereof calculated BLUEs are provided in Supplementary Table 1.

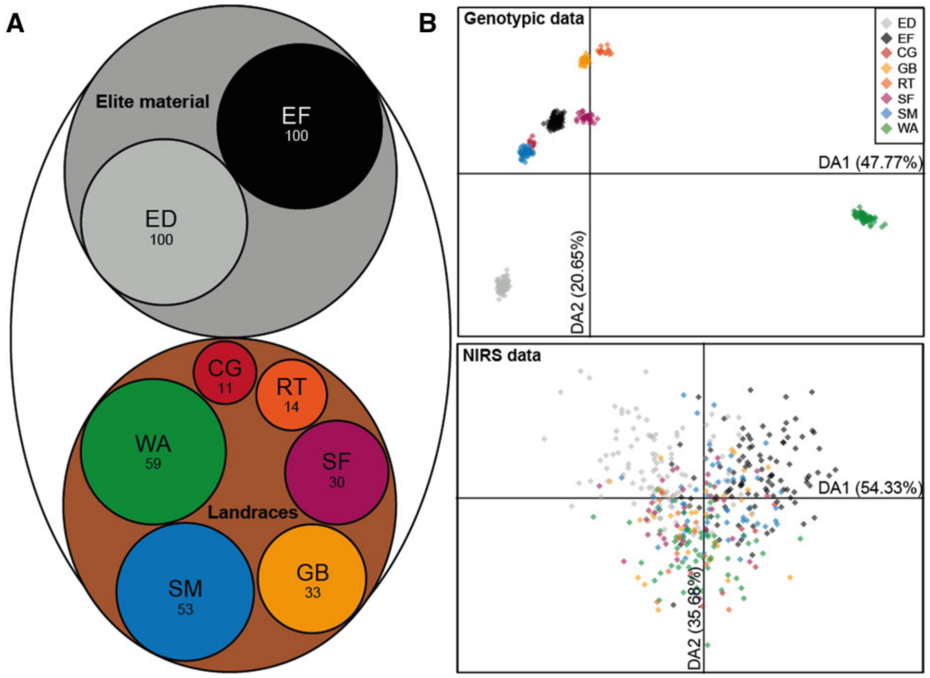

Fig. 1. Population composition and discriminant analysis of principal components (DAPC). a) Elite material ($n = 200$) with ED and EF, landraces ($n = 200$) as a whole (LR) with CG, GB, RT, SM, SF, and WA. The size of each group is represented by the circle size and given as the number of individuals. b) DAPC of all 400 individuals from the 8 groups, performed with marker data (top) and NIRS data (bottom). The amount of variance explained by the first two discriminant analysis functions DA1 and DA2 is given in brackets.

Genotypic data

All 400 genotypes were characterized with a 50K SNP array by Illumina (Ganal et al. 2011), resulting in total in 57,840 calls. Genotypic raw data are provided in Supplementary Table 2.

Quality control

As a first step, all marker data were filtered for their information content and 8,254 markers were found to only consist of missing values. The threshold of >50% for missing marker information and >5% for heterozygous state led to the exclusion of additional 844 markers and 637 markers, respectively. In addition, 2,222 monomorphic markers were detected of which 2,093 were not yet included in the missing and heterozygote filter steps and therefore also removed from the genotypic data, leaving 46,012 markers in the overall genotypic data. Next, we checked that no individual genotype fell under the criteria of having >20% missing marker data and/or >5% heterozygous markers. No filtering of genotypes due to these criteria was necessary. Finally, the genotypic data were split up according to the elite dent ($n = 100$), elite flint ($n = 100$), and landraces ($n = 200$) categorization. In each of these groups, markers containing only missing and/or monomorphic markers, and markers with a minor allele frequency (MAF) of <3% were once again removed from the subsets. Heterozygous marker information was in contrast to the complete marker data set not excluded but instead set to NA. All these filtering steps led to a total of 34,145 markers for the ED, 33,422 markers for the EF, and 38,284 markers for the LR group.

Imputation

The 3 groups were kept separately for imputation. Therefore, all 3 files were loaded into TASSEL (Bradbury et al. 2007) in HapMap format and transformed to the Variant Call Format. This format was then used for imputation by the software Beagle 5.2 (Browning et al. 2018). Standard settings for the imputation procedure were chosen, except for the effective population size n_e, which was reduced to 1,000. After successful imputation, all 3 files were filtered and markers with MAF <5% were removed. The so obtained files were then merged, which resulted in a total of 17,845 imputed markers for the investigated 400 genotypes.

Genomic selection

We performed genomic selection with the R-package "rrBLUP" 4.6.1 with the basic model written as: $y = WGu + \varepsilon$ (Endelman 2011), where the phenotypic value y is calculated by the product of the design matrix W, the genotype matrix G, and the vector u of marker effects with ε being the error term. Ridge regression keeps all markers in the model and shrinks their estimated effects by a constant factor (Whittaker et al. 2000). In general, we can distinguish between *predictions within* a group, where 5-fold cross-validation was performed and *predictions among* groups without cross-validation. Single landrace populations were only considered for ≥30 individuals. To perform 5-fold cross-validation, the dataset was for each run divided randomly into 80% training set and 20% prediction set. For the heterogeneous group "landraces", consisting of 6 single populations, proportional sampling was performed per landrace and then the training set and the prediction set were combined accordingly. For within-group predictions, the number of cross-validation runs was 1,000, if not mentioned otherwise. The predictive ability was calculated as the Pearson correlation between predicted values and the BLUEs of each prediction set.

NIRS data

NIRS data were obtained with a SpectraStar (Unity Scientific, Milford, MA, USA). The device covers the wavelength range between 1,250 and 2,400 nm with a stepsize of 1 nm (NIRS raw data are given in Supplementary Table 3 and are depicted in Supplementary Fig. 2a). In addition to seed samples from all 3 environments, NIR spectra of seedling biomass samples grown in EWE_2020 were recorded. Seed samples were obtained from ca. 375 g of the open-pollinated harvest sample used for the determination of grain dry matter content. All samples were ground to a final fineness of 1 mm filling a 200-ml tube (RETSCH GmbH, Haan, Germany). Of these subsamples, one NIR cup per plot was assembled and measured with 24 repetitions.

Quality control

First, the NIR spectra were cut at each side of the spectra by 18 nm to exclude undesired border effects, leaving 1,114 wavelengths to be further analyzed. Subsequently, Savitzky-Golay smoothing and first derivative of the NIR spectra were applied by means of the R-package "prospectr" 0.2.0 (Stevens and Ramirez-Lopez 2020). Afterwards, starting from wavelength 1,268 to wavelength 2,382, BLUEs were calculated of each single wavelength following the mixed model denoted in Equation (1). Heritabilities per wavelength were calculated (Supplementary Fig. 2b) and variance components per wavelength checked (Supplementary Fig. 2c).

Phenomic selection

All NIRS BLUEs were centered and scaled using the function "scale" in R before the so obtained values were subjected to further analyses. The standardization of the data is paramount for assuming a common variance of the regression coefficients (Piepho 2009). Exactly the same procedure as described for genomic prediction was carried out for phenomic prediction, except for using NIR data instead of marker data. Again, within group predictions were performed with 1,000 cross-validation runs. For predictions within and among groups, the two smallest populations with <30 individuals, namely CG and RT were not considered as a separate group. Hence, the following 7 groups were used for all genomic and phenomic predictions: elite Dents (ED), elite Flints (EF), landraces as a whole (LR) as well as the single landraces Gelber Badischer (GB), Satu Mare (SM), Strenzfelder (SF), and Walliser (WA).

If not specified otherwise, we used RStudio version 3.5.3 for all described data analyses (R Studio Team 2020) and the R packages "ggraph" (Lin Pedersen 2021), "ggpubr" (Kassambara 2020), "adegenet" (Jombart 2008) as well as basic R plot functions.

Results

Characterization of the material groups by phenotypic, genotypic, and NIRS data

The 400 genotypes that were investigated in this study can be differentiated into 8 groups: 100 elite lines of each of the two heterotic groups Flint and Dent, and 200 lines from 6 European Flint landraces (Fig. 1a). The discriminant analysis of principal components (DAPC) showed that the marker data reflected this underlying population structure, whereas no distinct clustering of the groups was apparent with NIR spectra (Fig. 1b).

Furthermore, we assessed the phenotypic variation present in the plant material (Fig. 2 and Supplementary Table 4). This revealed that the single landraces exhibited significant differences between each other for certain traits and can therefore also phenotypically not be considered as one homogeneous group. As expected, the elite material showed on average significantly higher yields than the landraces. Overall, it could be observed that the higher the grain yield was, the lower were the P concentrations in the kernels. Notably, the elite Dent lines are generally later maturing under European field conditions than the elite Flint lines, which explains the grain dry matter content values and may also underlie their observed lower P concentrations in the maize kernels. Broad-sense heritabilities for the multienvironment analysis were overall high, ranging from 0.48 for grain dry matter content to 0.97 for final plant height, both in the landrace CG (Fig. 2 and Supplementary Table 4). Moreover, the genotypic variance components in each group illustrated the larger variation of landraces compared to elite material for the traits anthesis-silking interval, early vigor, and final plant height. For the trait grain yield, it was the other way around, with the broadest variation observed in elite material.

When correlating the BLUEs of the phenotypic data with those of the NIR spectra, we observed rather low correlations for most wavebands and different correlation patterns for the different traits (Supplementary Fig. 2d). Interestingly, opposed correlations for grain yield and P concentration with the NIR reflectance values were observed. Collectively, these results underpin that molecularly and phenotypically diverse breeding material was represented in this panel, which is therefore well suited to address the objectives of this study.

Predictions within groups

We first performed genomic and phenomic prediction within groups (Fig. 3). This revealed no consistent pattern, as for most traits and groups the phenomic and genomic predictive ability was comparable, with sometimes one or the other being better, but often only slightly. Genomic prediction achieved generally better results for the traits final plant height and grain dry matter content. Conversely, grain yield was overall better predicted by phenomic prediction and for the trait P concentration phenomic prediction outperformed genomic prediction substantially for all groups. The predictive ability values obtained by cross-validation, confirmed for genomic and phenomic prediction alike that smaller groups (the landraces GB, SM, SF, and WA), and therefore smaller training and prediction sets, resulted in lower mean predictive abilities with a generally larger variation.

We then focused on the cases where we observed the most prominent discrepancies between the genomic and the phenomic prediction approach in the 3 major groups. Large differences between both approaches were observed for predictions in the group of landraces. For early vigor, genomic prediction outperformed phenomic prediction by 0.46. On the other hand, P concentration was characterized by a 0.40 higher phenomic predictive ability compared to genomic prediction. In order to illustrate the cause for these differences, we plotted for 10 cross-validation runs the predicted and observed values of the traits early vigor and P concentration for each individual genotype of the 6 landraces (Fig. 4). For early vigor, the overall correlation coefficient of the genomic approach was relatively high with $r = 0.69$. However, the mean trait performance was quite different among the landraces and the correlations assessed within them only averaged to $\bar{r} = 0.03$. This low predictive ability within each landraces was reflected by phenomic prediction, for which the overall correlation coefficient r was indeed low with 0.05, hence only slightly deviating from the average $\bar{r}$ across the landraces that was 0.13 and thus even higher compared to the genomic approach ($\bar{r} = 0.03$). In the case of P concentration for which phenomic prediction yielded a much higher predictive ability than genomic prediction, the genomic approach resulted in low within-landrace correlation coefficients ($\bar{r} = -0.19$), but also a low overall correlation ($r = 0.28$), as the phenotypic differences among the landraces were minor. By contrast, phenomic prediction showed a high overall correlation coefficient of $r = 0.79$,

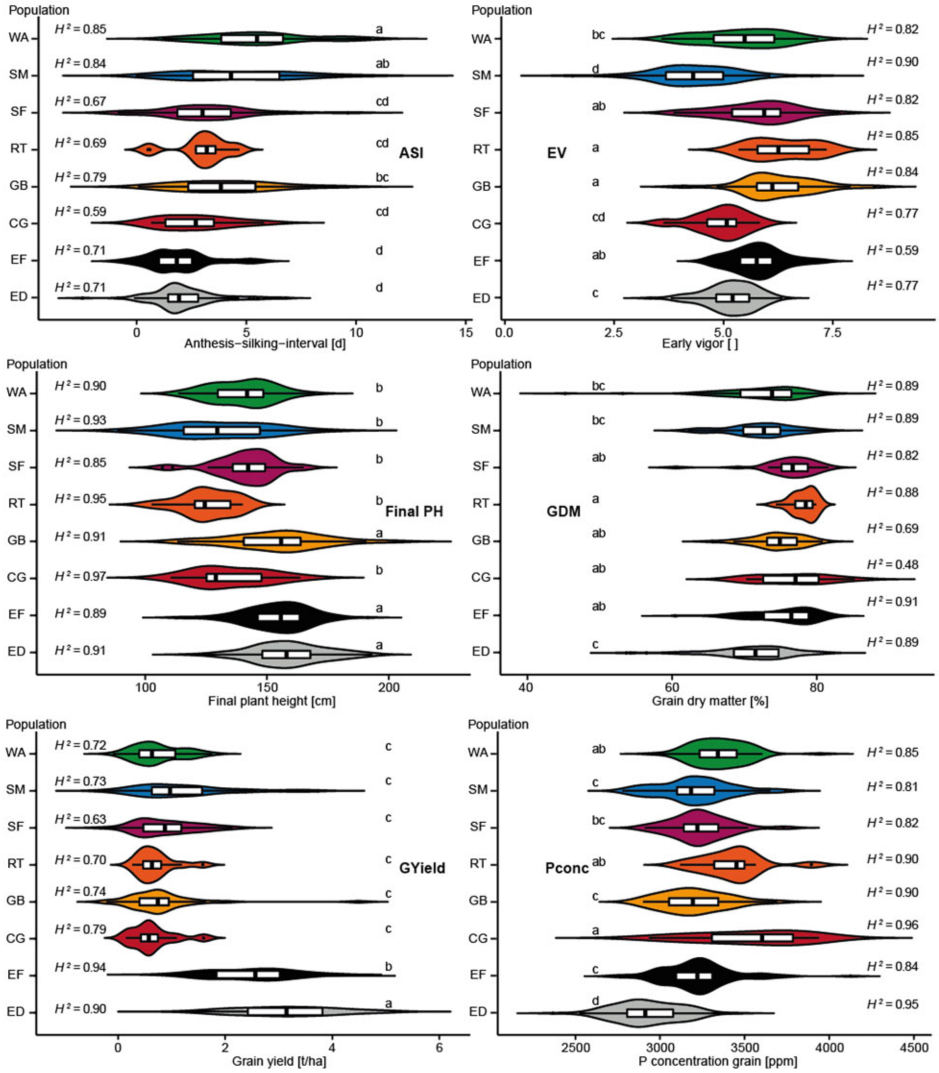

Fig. 2. Phenotypic variation in the 8 groups. Groups are abbreviated as WA, SM, SF, RT, GB, CG, EF, and ED. Distribution of the phenotypic trait values for ASI (anthesis-silking-interval), EV (early vigor), Final PH (plant height at harvest), GDM (grain dry matter content), GYield (grain yield), and Pconc (phosphorus concentration in kernels) shown per group. The letter display indicates significant differences of the means; groups with the same letter are not significantly different from each other ($\alpha = 0.05$). H^2 denotes the broad-sense heritability.

which was again mirrored by the correlation coefficients observed in each single landrace ($\bar{r} = 0.72$).

Predictions among different groups

We next assessed predictions among groups, i.e. using one group as training set and another one as prediction set (Fig. 5 and Supplementary Figs. 3 and 4). In general, the phenomic predictive abilities surpassed the genomic ones when one of the 3 major groups was used as training set. An exception was the prediction of final plant height when using elite Dents as training set, as for this scenario the predictive ability was negative for prediction in all other groups, whereas positive predictive abilities were achieved for the genomic approach except for the elite Flints. However, especially for the traits grain dry matter content, grain yield, and P concentration, phenomic prediction yielded substantially higher predictive abilities compared to genomic prediction. The same trends as observed for the 3 major groups, though potentially slightly less pronounced, were observed when the two largest landraces, SM or WA, were used as training set (Supplementary Fig. 3). Phenomic prediction yielded overall more stable results, which can also be well seen in the visualization of the predictive abilities separated by group (Supplementary Fig. 4). Here, we can also conveniently compare reciprocal predictions, which substantiated the much lower robustness of genomic compared to phenomic among-group predictions. The phenomic prediction results, by contrast, yielded similar patterns no matter in which direction the prediction took place.

Phenomic predictions were also performed with NIR spectra of maize seedling biomass for the environment EWE_2020. Predictions based on biomass generally achieved lower predictive abilities than seed-based ones. This held true for the comparisons of the seed NIRS BLUEs across all 3 environments (results not shown) as well as for the seed NIRS data of EWE_2020 alone (Supplementary Table 5).

In summary, phenomic prediction resulted in much higher predictive abilities than genomic prediction for the prediction among groups.

Evaluation of composite training sets

The trait grain yield was chosen to investigate the potential of combining groups into composite training sets, which were then larger but also composed of material from different

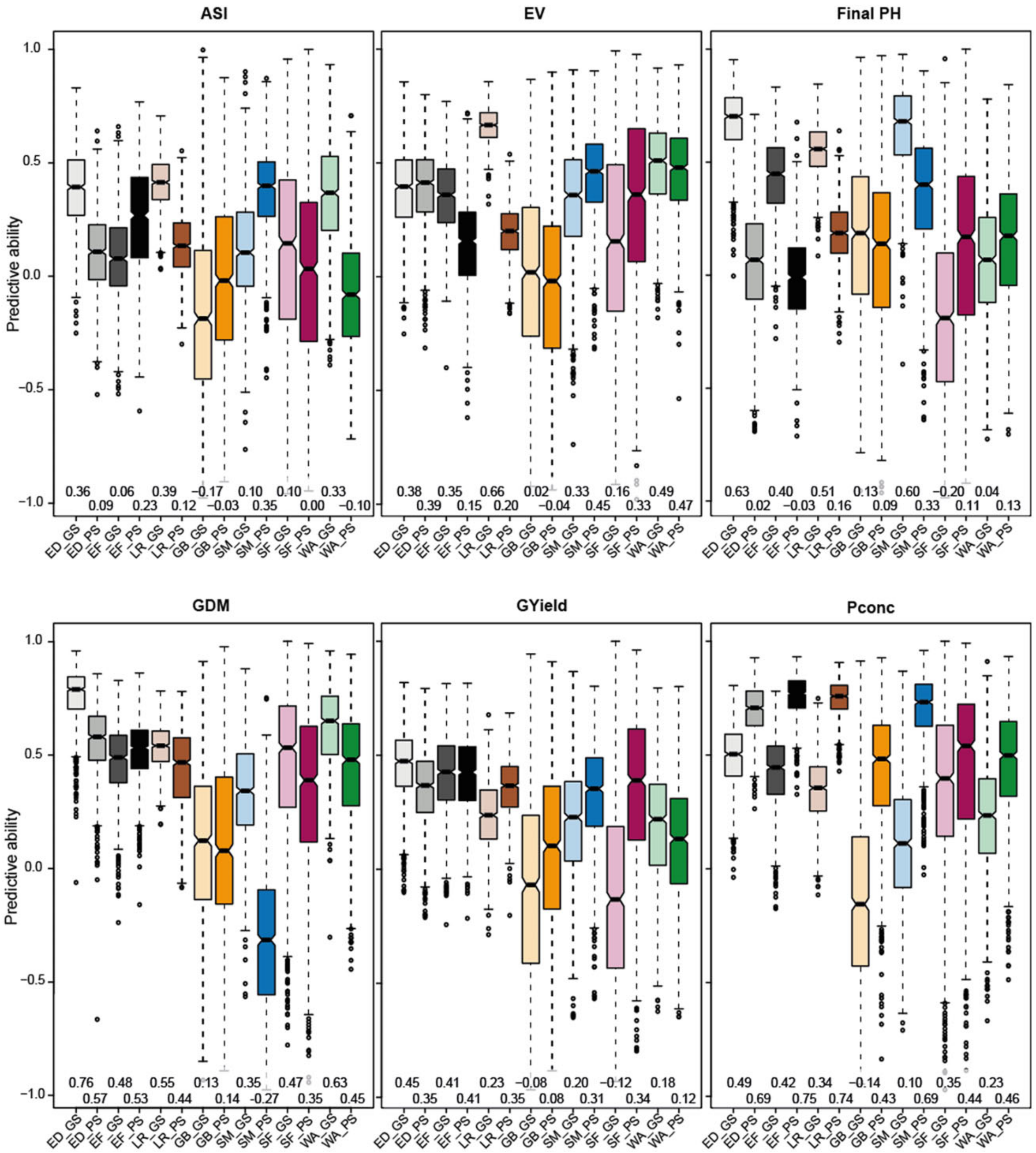

Fig. 3. Multiple comparisons of the predictive abilities by within-group prediction. Cross-validated predictive abilities obtained by 1,000 runs are shown for each trait and group. Groups are abbreviated as ED, EF, all LR, GB, SM, SF, and WA. GS and PS abbreviate genomic and phenomic prediction, respectively. The mean of each scenario is given underneath the boxplots. Traits are denoted as ASI, EV, Final PH, GDM content, GYield, and Pconc.

groups with different trait performance (Fig. 6). To predict elite Dents and elite Flints, the following scenarios were compared with each other: (1) 5-fold cross-validated prediction within the respective elite group, (2) across prediction from the other elite group as well as from the landraces group as a whole, and (3) combinations of 80% of the lines from the elite group to be predicted with one or both of the other major groups to predict the 20% remaining lines of the respective elite group. Phenomic prediction resulted in highly similar predictive abilities for all 6 scenarios for both the elite Flint and elite Dent lines. As shown before, genomic prediction was on a comparable level for the cross-validated within-group prediction, but performed poorly for the among-group prediction. Interestingly, genomic prediction then resulted in similar or even slightly higher predictive abilities compared to phenomic prediction for the 3 composite training sets. The predictive abilities achieved with these composite training sets were similar to that obtained by the within-group prediction.

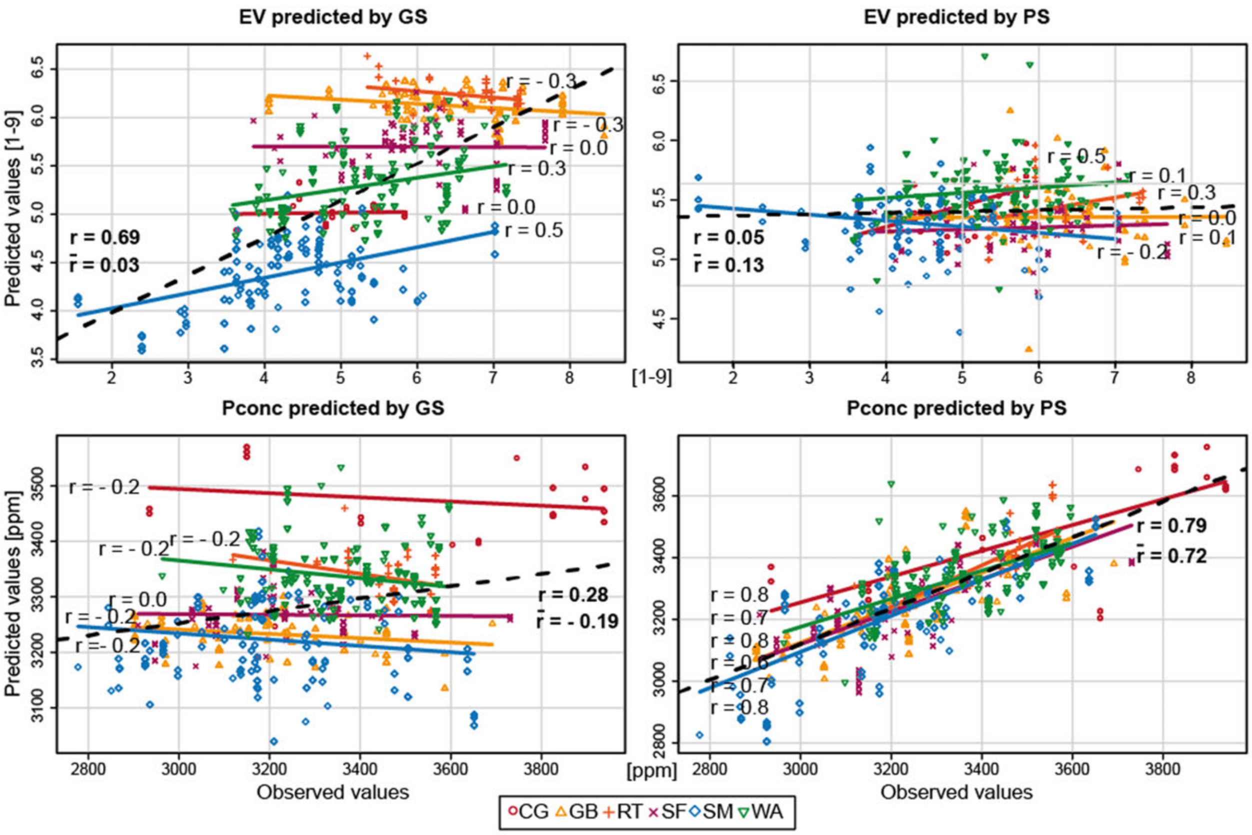

Fig. 4. Predictive ability in the heterogenous group of landraces. Comparison of genomic (GS) and phenomic (PS) prediction for the traits EV and Pconc. For EV, the genomic predictive ability was much higher, whereas for Pconc the phenomic approach resulted in a much higher predictive ability than genomic prediction. The Pearson correlation coefficients within each group are indicated as r. In bold, the Pearson correlation coefficient across all genotypes is given and $\bar{r}$ denotes the mean of all correlation coefficients of the single landraces. The dots represent the observed and the predicted trait values from 10 cross-validation runs.

Discussion

Comparison of genomic and phenomic prediction

First, it has to be stated that a high quality of phenotypic data is and will remain the basis for all breeding activities (Bernardo 2021a). This was given in this dataset, which showed a large genotypic variation and high to very high trait heritabilities in the multienvironment analysis (Supplementary Table 4).

Looking at the obtained cross-validated predictive abilities within groups, genomic and phenomic prediction generally yielded comparable results (Fig. 3). This performance was, however, also dependent on the trait (Knoch et al. 2021) and the genetic group. It should be mentioned here, that no general conclusion should be drawn solely from the results of the single landraces with their rather small population sizes. Final plant height, for example, was much better predicted by marker than by NIRS data, but mainly in the elite material. This material is somewhat taller than the landrace lines, but otherwise there is no apparent difference. For grain dry matter content, the difference in predictive ability was most pronounced for the elite Dents and the landrace SM from Romania, which are the two latest maturing groups as can be seen by their lowest means for this trait (SM = 72.81%, ED = 70.88%). All seed samples were completely dried before NIR spectra were measured and one might assume that drying maize kernels containing more water at the beginning might change their properties in a way that altered the NIRS assessment. However, for other traits like early vigor or grain yield, phenomic prediction was as good or even better than genomic prediction for these two groups. For grain yield, we found that both approaches performed similarly, which is promising as grain yield is a central trait in every breeding program. In line with this, Lane et al. (2020) reported prediction abilities above 0.7 for grain yield obtained by phenomic prediction of whole-kernel maize samples. For the trait P concentration, we observed higher phenomic predictive abilities compared to the genomic approach for all groups. Notably, P concentration is also an endophenotype of the seeds, which may have contributed to this performance.

Taken together, the reasons for the discrepancies observed between the two approaches for some traits and groups are not clear and require further research. Nevertheless, our results confirmed the potential of phenomic prediction for NIRS-assisted selection in breeding, as the phenomic predictive abilities were generally competitive with those from genomic prediction. NIRS data can already be obtained from early- or mid-generation selection candidates before any yield trials in multiple environments have been performed. Phenomic prediction can thus be used to predict more resource-intensive traits such as grain yield and thereby assist the identification of the most promising candidates to be advanced to the next generation.

Population structure can lead to overestimation of the genomic predictive ability

The greatest differences between the genomic and phenomic predictive abilities were often observed for the panel of landraces, where for the 3 traits anthesis-silking interval, early vigor, and

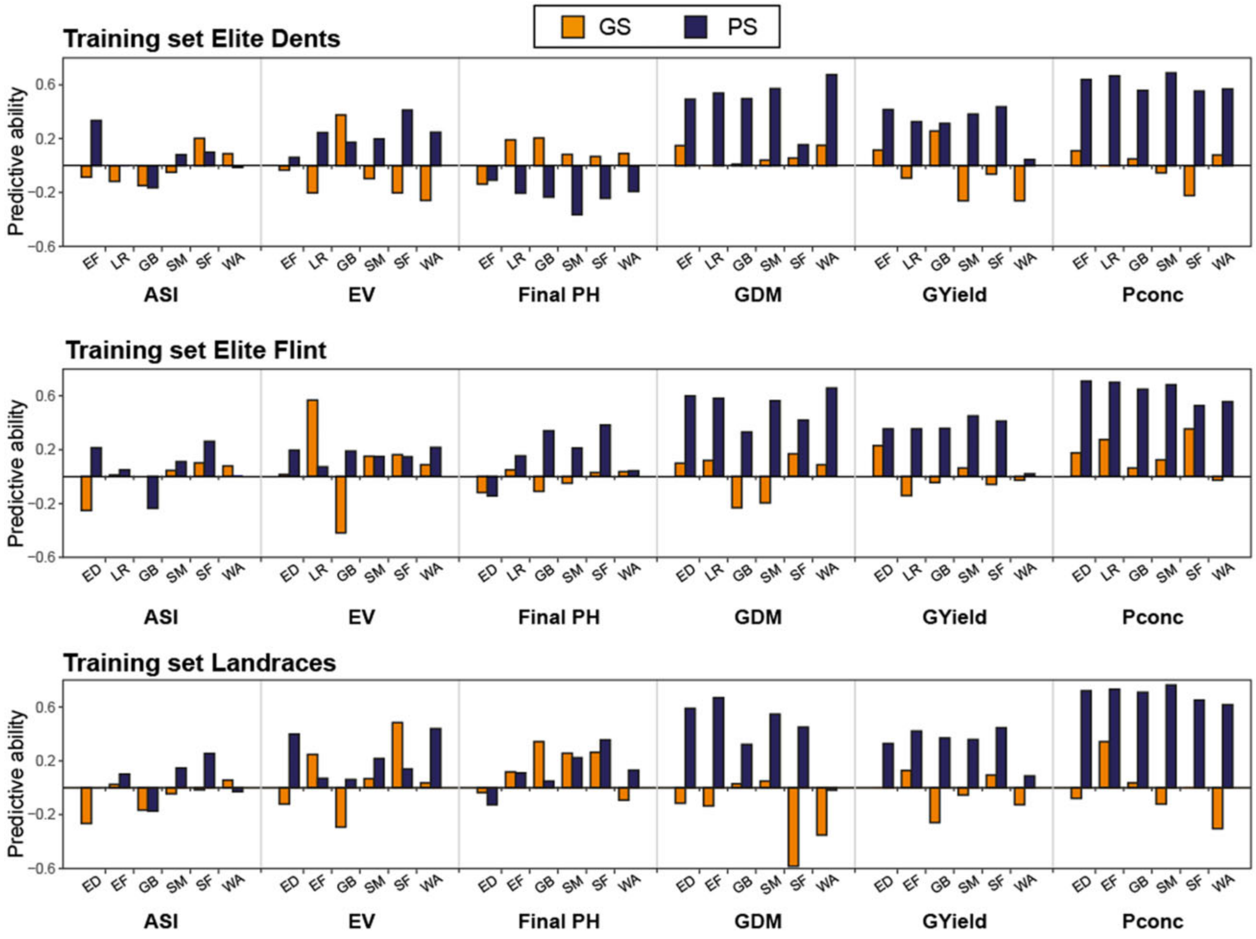

Fig. 5. Predictive ability of among-group predictions. Results are shown for the ED, EF or the landraces being used as training set for genomic (GS) or phenomic (PS) prediction to predict each of the other groups. Groups are abbreviated as ED, EF, all LR, GB, SM, SF, and WA. Traits are denoted as ASI, EV, Final PH, GDM content, GYield, and Pconc.

final plant height genomic prediction appeared to perform much better. We exemplarily used early vigor to further investigate this different performance. Analyzing the correlations between the observed and the predicted trait values per landrace revealed that the high predictive ability of early vigor by genomic prediction was an artifact (Fig. 4). While the overall correlation coefficient was high with 0.69, each single landrace showed only weak correlations that averaged 0.03. The reason for this is the confounding of population structure and trait performance, here in the form of the different landraces and their mean performance, a phenomenon which has been described in previous studies (Windhausen et al. 2012). All 3 traits showed clear differences in their means among the landraces (Fig. 2). In addition, the marker data can clearly separate landraces as shown by the discriminant analysis of principal components (Fig. 1B). In contrast to the marker data, the NIRS data do not distinguish the LR as groups in the DAPC as clearly as the marker data and are therefore less prone to this kind of artifact. For the NIRS data, the low overall correlation coefficient for early vigor of 0.05 much more accurately reflected the predictive ability in the single landraces.

For the reverse case of phenomic prediction outperforming genomic prediction in the landraces for the trait P concentration, by contrast, the high overall correlation coefficient of the former correctly portrays the high predictive ability in each of the landraces. In summary, this confirms previous results concluding that genomic selection is sensitive to population structure (Thorwarth et al. 2017). As a consequence, seemingly high genomic predictive abilities achieved with panels showing population structure should be interpreted with caution and always in combination with the trait performance of the populations.

Phenomic prediction works well among different breeding material

A major advantage of phenomic prediction became apparent when predicting from one group to another (Fig. 5). Genomic prediction has been described to strongly depend on the relatedness between training and prediction set (Albrecht et al. 2011; Riedelsheimer et al. 2013; Li et al. 2021; Zhu et al. 2021). Our results corroborate these findings, as the prediction among groups resulted in only low predictive abilities, even for the predictions among the more closely related Flint material. The phenomic predictive abilities, on the contrary, were much higher, especially for the traits grain dry matter content, grain yield, and P concentration. For these 3 traits, the among-group predictive abilities were often as high as the cross-validated within-group ones.

Collectively, these findings illustrate that phenomic prediction is very promising for rather diverse breeding material with more or less unrelated groups, whereas genomic prediction has been shown to work best if the material in the training and prediction set are from the same group (Schopp et al. 2015).

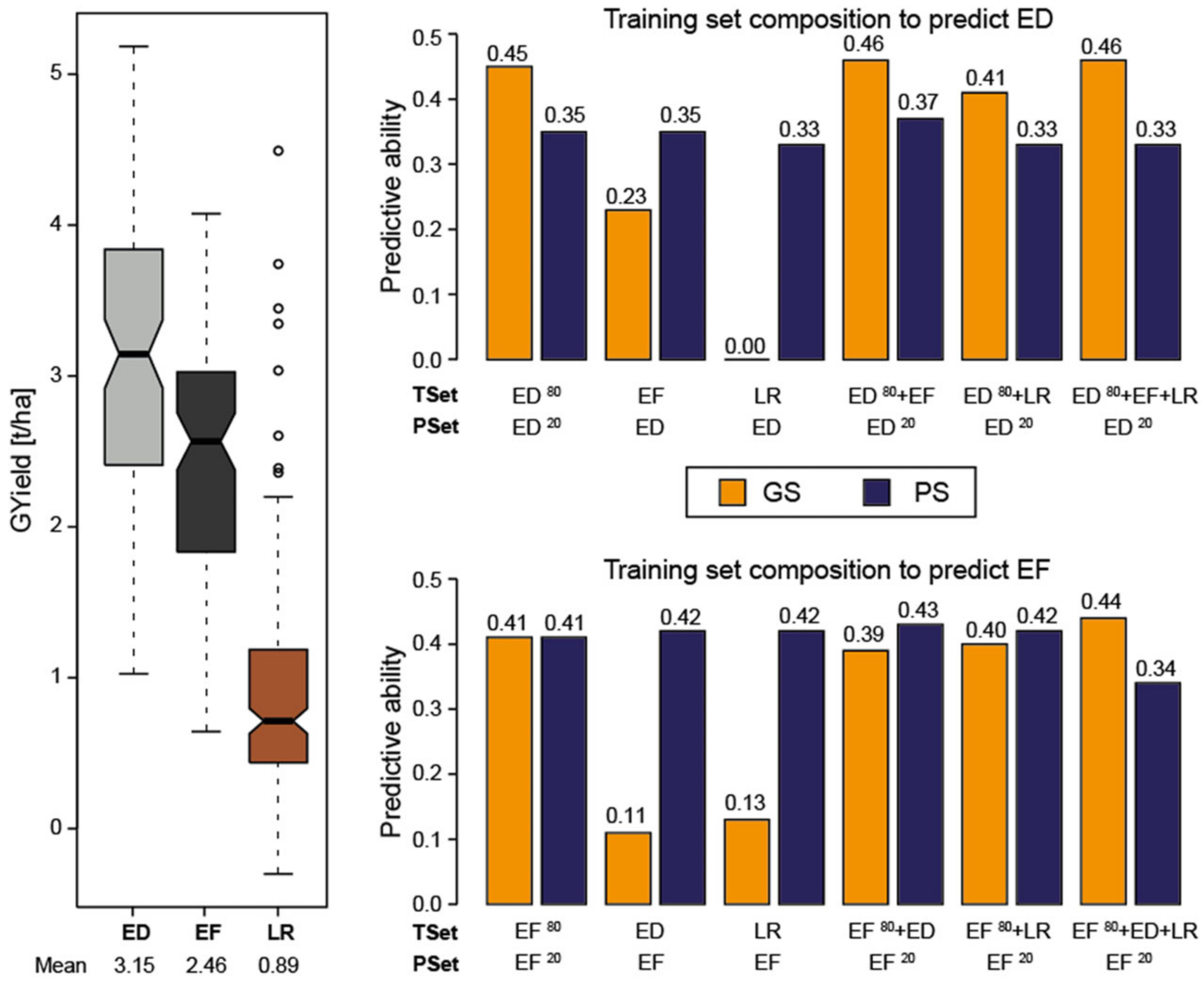

Fig. 6. Evaluation of composite training sets. For the 3 major groups, ED, EF, and landraces, different training set compositions were tested for grain yield for genomic (GS) and phenomic (PS) prediction. The boxplot on the left shows the differing level of phenotypic performance for the 3 groups. The bar plots on the right depict the predictive ability of the different scenarios for either the ED (top) or the EF (bottom). The superscript numbers 80 and 20 reflect the proportion of individuals in the training set (TSet) and the prediction set (PSet). Predictions within the respective elite groups were obtained with 1,000 5-fold cross-validation runs; for the composite training sets, 100 cross-validation runs were used.

Composition of the training set with diverse breeding material

These findings rose the question on how different compositions of the training set would affect the prediction of grain yield of the elite lines. Notably, grain yield differed substantially between the elite material and the landraces. As phenomic prediction appeared to be tolerant to unrelated material being used as training set, we hypothesized that increasing the training set with lines from other material groups than the one to be predicted would improve or at least not hamper the predictive ability of phenomic prediction. While the other elite group and the landraces both yielded predictive abilities similar to the cross-validated within-group phenomic predictions, adding them to the training set did not enhance the predictive ability. For the prediction of both elite groups, the phenomic predictive abilities were more or less unchanged for all tested scenarios of training set composition. It might be that even though the predictive abilities of all 3 groups are highly similar, the effect estimates are different and combining them does not yield an advantage or that even with the smallest training set size of the 80 lines (80%) sampled from the same group, the predictive ability already reached a plateau stage. Our results of phenomic predictive abilities between 0.33 and 0.43 with combined training sets are consistent with a former study, which reported a phenomic prediction ability based on maize kernel NIRS of on average 0.28 for grain yield in elite material, when a diversity set and 10% of each group to be predicted were used as training set (Lane et al. 2020).

While the genomic prediction using one of the other two major groups virtually failed, adding them to the training set resulted in similar or even slightly higher predictive abilities as the cross-validated within-group predictive ability. This is in line with previous findings that showed that adding less related lines to a training set did not reduce the predictive ability (e.g. Brauner et al. 2020; Li et al. 2021; Zhu et al. 2021) and increasing the training set size generally results in higher predictive abilities of genomic selection (e.g. Zhao et al. 2012; Thorwarth et al. 2017; Li et al. 2021; Zhu et al. 2021).

Nevertheless, if all scenarios are considered, phenomic prediction showed a higher robustness of the predictive abilities for different compositions of the training set and thus relatedness between training and prediction set. This result is also worth mentioning as it suggests that the general assumption that predictive breeding strongly relies on estimating the genetic relatedness among individuals (Bernardo 2021a) may not hold true for the approach of phenomic prediction.

Possible effects of the NIRS sample material on the trait prediction

Spectral data can not only be obtained from seeds but also from other plant material, thereby potentially allowing selection at different stages of a breeding program. We derived NIR spectra from maize kernels as well as for one environment from seedling biomass samples, both ground to 1 mm. In our study, the results obtained with the seed sample NIRS data were generally better (Supplementary Table 5). This is in line with previous findings that in addition showed higher genotypic variances for grain in comparison to leaf samples, which may underlie the higher predictive abilities of the former (Rincent et al. 2018). Interestingly, when looking at the single traits separately, we observed higher predictive abilities based on NIRS of seedling biomass for early vigor and final plant height, whereas anthesis-silking-interval, grain dry matter content, grain yield and P concentration were clearly better predicted by seed samples. The picture this presents is that the closer the sampled tissue is in relation to the trait of interest, the better the prediction works based on this tissue. Our data set is clearly too small to substantiate this conclusion, but this warrants further research. We also correlated the NIRS BLUEs of the seedling biomass with the trait BLUEs for each genotype, but could not discern a pattern between these correlations and the goodness of the trait prediction (data not shown). It should be noted here that grain yield is often the most important trait for breeders and therefore seed-based NIR spectra appear more promising for application in breeding programs.

Application of phenomic prediction in practical breeding

Rincent et al. (2018) showed in simulations that for different scenarios regarding the costs and reliabilities of phenomic and genomic prediction, the expected selection gain was in most cases higher for phenomic compared to genomic selection. The advantages of performing predictions based on NIRS data compared to genotypic data are the low requirements for infrastructure such as specialized laboratories and the strongly reduced costs, both coupled with the benefit of an increased speed and efficiency of selection in the breeding program. The first point challenges the assumption that became prevalent in the last years, namely that the most cost-efficient tool for trait prediction is found in genetic marker data (Bernardo 2021b; Knoch et al. 2021). Supposed that seeds represent the most suitable material to obtain NIR spectra, we are much faster to capture all data with a state-of-the-art spectrometer compared to the DNA extraction and subsequent genotyping. If we have to take decisions that are time-critical, as for example, frequently encountered in winter cereals between harvest and sowing or in maize in between shuttle breeding seasons, phenomic prediction results can be obtained quicker than genomic prediction results. Moreover, the phenomic selection approach appears particularly attractive for comparatively low-tech institutions because less investments and resources are needed for NIRS measurements compared to genotyping.

A main finding of this study is that phenomic prediction also reliably works for designs of training sets that show a population structure as well as for the case that the training set and the prediction set include less related material. In breeding programs that stay within their established material, as for example in maize within the existing heterotic groups, this is of no relevance. So, when could this become relevant in practical breeding? For instance, if we were to start a breeding program in a new environment by combining material from different origin. After an initial field evaluation and yield trials with the labor- and/or resource-intensive traits being assessed only on a subset of lines, phenomic prediction based on the NIR spectra of the harvested material of all lines could support the identification of further candidates to be more intensively tested. Likewise, broadening the genetic basis of a breeding program by introgression of less related material from different groups, as exemplified here with the landraces, will require testing this material but may also profit from the additional prediction of the expected performance. Furthermore, crops with yet undefined heterotic pools or the presence of subpopulations such as wheat (Boeven et al. 2016) or sorghum (da Silva et al. 2021) could benefit from the independence of the performance of phenomic prediction with regard to the training set composition and its possibly underlying population structure. In addition, breeding programs are driven by the different selection cycles, which by nature imply a decreasing relatedness of the individuals from one cycle to the next (Schopp et al. 2015; Auinger et al. 2021) . Further research is required to investigate whether phenomic prediction can provide higher predictive abilities than genomic prediction when using the current cycle as training set for the prediction of the individuals of the next cycles. Eventually, the decision of whether to use the genomic or the phenomic approach will be made in very practical terms, depending on the available resources and infrastructure, as well as the characteristics of the particular breeding program.

Conclusion

While a large number of studies is available on different aspects of genomic prediction, the use of NIRS or other spectral data for phenomic prediction is still in its beginnings. We therefore compared both approaches under different scenarios for a set of 6 traits relevant in maize breeding. Apart from being cost-efficient and amenable to high-throughput, the potential of phenomic selection lies in its reliable predictions also under structured training sets as well as for predictions into unrelated material. In the end, however, it is not about a competition of the two approaches, but rather to expand the breeder's toolbox and ideally one can choose from different approaches the one that is best suited for a given situation. Thus, resources can be allocated in the best possible way in order to maximize selection gain. Just as seen for genomic prediction over the past years, further research is required to better understand and refine the approach of phenomic prediction toward a broader application in plant breeding. Specifically, NIRS sample material and the environmental effect on the spectra should be investigated in the future. Nevertheless, our results demonstrate the value of phenomic prediction as a low-cost and efficient tool to support selection of complex traits in plant breeding.

Data availability

The authors affirm that all data necessary for confirming the conclusions of this article are represented fully within the article and its tables and figures. Supplemental material is included at figshare: https://doi.org/10.25387/g3.16692409.

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Conflicts of interest statement

None declared.

Literature cited

Agrometeorology Baden-Württemberg. Monthly averages > Graph. Karlsruhe, Deutschland: landwirtschaftliches Technologiezentrum Augustenberg; 2021 [accessed 2021 Jul 8]. https://www.wetter-bw.de/Agrarmeteorologie-BW/Wetterdaten/Stationskarte.

Aguate FM, Trachsel S, Pérez LG, Burgueño J, Crossa J, Balzarini M, Gouache D, Bogard M, de los Campos G. Use of hyperspectral image data outperforms vegetation indices in prediction of maize yield. Crop Sci. 2017;57(5):2517–2524.

Albrecht T, Wimmer V, Auinger H-J, Erbe M, Knaak C, Ouzunova M, Simianer H, Schön C-C. Genome-based prediction of testcross values in maize. Theor Appl Genet. 2011;123(2):339–350.

Auinger H-J, Lehermeier C, Gianola D, Mayer M, Melchinger AE, da Silva S, Knaak C, Ouzunova M, Schön CC. Calibration and validation of predicted genomic breeding values in an advanced cycle maize population. Theor Appl Genet. 2021;134(9):3069–3081.

Benavente E, Giménez E. Modern approaches for the genetic improvement of rice, wheat and maize for abiotic constraints-related traits: a comparative overview. Agronomy. 2021;11(2):376.

Bernal-Vasquez A-M, Utz H-F, Piepho H-P. Outlier detection methods for generalized lattices: a case study on the transition from ANOVA to REML. Theor Appl Genet. 2016;129(4):787–804.

Bernardo R. Predictive breeding in maize during the last 90 years. Crop Sci. 2021a;61(5):2872–2881.

Bernardo R. Upgrading a maize breeding program via two-cycle genomewide selection: same cost, same or less time, and larger gains. Crop Sci. 2021b;61(4):2444–2455.

Boeven PHG, Longin FH, Würschum T. A unified framework for hybrid breeding and the establishment of heterotic groups in wheat. Theor Appl Genet. 2016;129(6):1231–1245.

Böhm J, Schipprack W, Utz HF, Melchinger AE. Tapping the genetic diversity of landraces in allogamous crops with doubled haploid lines: a case study from European flint maize. Theor Appl Genet. 2017;130(5):861–873.

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES. TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics. 2007;23(19):2633–2635.

Brauner PC, Müller D, Molenaar WS, Melchinger AE. Genomic prediction with multiple biparental families. Theor Appl Genet. 2020;133(1):133–147.

Browning BL, Zhou Y, Browning SR. A one-penny imputed genome from next-generation reference panels. Am J Hum Genet. 2018;103(3):338–348.

Butler DG, Cullis BR, Gilmour AR, Gogel BJ. ASReml-R Reference Manual: ASReml Estimates Variance Components under a General Linear Mixed Model by Residual Maximum Likelihood (REML). Australia: Queensland Department of Primary Industries; 2009. 160 p.

Cullis BR, Smith AB, Coombes NE. On the design of early generation variety trials with correlated data. JABES. 2006;11(4):381–393.

da Silva KJ, Pastina MM, Guimarães CT, Magalhães JV, Pimentel LD, Schaffert RE, de Oliveria Pinto M, de Souza VF, Bernardino K, Silva M, *et al.* Genetic diversity and heterotic grouping of sorghum lines using SNP markers. Sci Agric. 2021;78(6):335.

de Mendiburu F. agricolae: Statistical Procedures for Agricultural Research. R Package Version 1.3-2; 2020 https://cran.r-project.org/package=agricolae.

Endelman JB. Ridge regression and other kernels for genomic selection with R package rrBLUP. Plant Genome. 2011;4(3):250–255.

Galán RJ, Bernal-Vasquez A-M, Jebsen C, Piepho H-P, Thorwarth P, Steffan P, Gordillo A, Miedaner T. Integration of genotypic, hyperspectral, and phenotypic data to improve biomass yield prediction in hybrid rye. Theor Appl Genet. 2020;133(11):3001–3015.

Ganal MW, Durstewitz G, Polley A, Bérard A, Buckler ES, Charcosset A, Clarke JD, Graner E-M, Hansen M, Joets J, *et al.* A large maize (*Zea mays* L.) SNP genotyping array: development and germplasm genotyping, and genetic mapping to compare with the B73 reference genome. PLoS One. 2011;6(12):e28334.

Hallauer A, Filho JBM, Carena MJ. Quantitative Genetics in Maize Breeding. 3rd ed. New York (NY): Springer; 2010.

Hayes BJ, Panozzo J, Walker CK, Choy AL, Kant S, Wong D, Tibbits J, Daetwyler HD, Rochfort S, Hayden MJ, *et al.* Accelerating wheat breeding for end-use quality with multi-trait genomic predictions incorporating near infrared and nuclear magnetic resonance-derived phenotypes. Theor Appl Genet. 2017;130(12):2505–2519.

Heffner EL, Sorrells ME, Jannink J-L. Genomic selection for crop improvement. Crop Sci. 2009;49(1):1–12.

Heslot N, Yang H-P, Sorrells ME, Jannink J-L. Genomic selection in plant breeding: a comparison of models. Crop Sci. 2012;52(1):146–160.

Jiang S, Cheng Q, Yan J, Fu R, Wang X. Genome optimization for improvement of maize breeding. Theor Appl Genet. 2020;133(5):1491–1502.

Jombart T. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics. 2008;24(11):1403–1405.

Kassambara A. ggpubr: 'ggplot2' Based Publication Ready Plots. R Package Version 0.2.5; 2020 https://CRAN.R-project.org/package=ggpubr.

Knoch D, Werner CR, Meyer RC, Riewe D, Abbadi A, Lücke S, Snowdon RJ, Altmann T. Multi-omics-based prediction of hybrid performance in canola. Theor Appl Genet. 2021;134(4):1147–1165.

Krause MR, González-Pérez L, Crossa J, Pérez-Rodríguez P, Montesinos-López OA, Singh RP, Dreisigacker S, Poland J, Rutkoski J, Sorrells M, *et al.* Hyperspectral reflectance-derived relationship matrices for genomic prediction of grain yield in wheat. G3 (Bethesda). 2019;9(4):1231–1247.

Lane HM, Murray SC, Montesinos-López OA, Montesinos-López A, Crossa J, Rooney DK, Barrero-Farfan ID, de la Fuente GN, Morgan CLS. Phenomic selection and prediction of maize grain yield from near-infrared reflectance spectroscopy of kernels. Plant Phenome J. 2020;3(1):e0117737.

Li D, Xu Z, Gu R, Wang P, Xu J, Du D, Fu J, Wang J, Zhang H, Wang G. Genomic prediction across structured hybrid populations and environments in maize. Plants (Basel). 2021;10(6):1174.

Lin Pedersen T. An Implementation of Grammar of Graphics for Graphs and Networks. 2.0.5: RStudio, PBC; 2021 [accessed 2021 Jul 28]. https://ggraph.data-imaginist.com, https://github.com/thomasp85/ggraph.

Melchinger AE, Schmidt GA, Geiger HH. Evaluation of near infra-red reflectance spectroscopy for predicting grain and stover quality traits in maize. Plant Breed. 1986;97(1):20–29.

Meuwissen THE, Hayes BJ, Goddard ME. Prediction of total genetic value using genome-wide dense marker maps. Genetics. 2001; 157(4):1819–1829.

Montesinos-López OA, Montesinos-López A, Crossa J, de los Campos G, Alvarado G, Suchismita M, Rutkoski J, González-Pérez L, Burgueño J. Predicting grain yield using canopy hyperspectral reflectance in wheat breeding data. Plant Methods. 2017;13:4.

Parmley K, Nagasubramanian K, Sarkar S, Ganapathysubramanian B, Singh AK. Development of optimized phenomic predictors for efficient plant breeding decisions using phenomic-assisted selection in soybean. Plant Phenomics. 2019;2019:5809404.

Piepho H-P. Ridge regression and extensions for genomewide selection in maize. Crop Sci. 2009;49(4):1165–1176.

Piepho H-P, Möhring J. Computing heritability and selection response from unbalanced plant breeding trials. Genetics. 2007;177(3): 1881–1888.

Pollard K, Dudoit S, van der Laan MJ. Multiple testing procedures: R multitest package and applications to genomics. In: Gentleman R, Carey VJ, Huber W, Irizarry RA, Dudoit S, editors. Bioinformatics and Computational Biology Solutions Using R and Bioconductor. Statistics for Biology and Health Series. New York, NY: Springer; 2005. p. 251–272. https://doi.org/10.1007/0-387-29362-0_15.

Resende M, Muñoz P, Resende M, Garrick DJ, Fernando RL, Davis JM, Jokela EJ, Martin TA, Peter GF, Kirst M. Accuracy of genomic selection methods in a standard data set of loblolly pine (*Pinus taeda* L.). Genetics. 2012;190(4):1503–1510.

Riedelsheimer C, Czedik-Eysenberg A, Grieder C, Lisec J, Technow F, Sulpice R, Altmann T, Stitt M, Willmitzer L, Melchinger AE. Genomic and metabolic prediction of complex heterotic traits in hybrid maize. Nat Genet. 2012;44(2):217–220.

Riedelsheimer C, Endelman JB, Stange M, Sorrells ME, Jannink J-L, Melchinger AE. Genomic predictability of interconnected biparental maize populations. Genetics. 2013;194(2):493–503.

Rincent R, Charpentier J-P, Faivre-Rampant P, Paux E, Le Gouis J, Bastien C, Segura V. Phenomic selection is a low-cost and high-throughput method based on indirect predictions: proof of concept on wheat and poplar. G3 (Bethesda). 2018;8(12):3961–3972.

R Studio Team. RStudio: integrated Development Environment for R. 3.5.3. Boston, (MA): RStudio, PBC; 2020. http://www.rstudio.com/ [accessed 2022 January 13].

Schopp P, Müller D, Technow F, Melchinger AE. Accuracy of genomic prediction in synthetic populations depending on the number of parents, relatedness, and ancestral linkage disequilibrium. Genetics. 2017;205(1):441–454.

Schopp P, Riedelsheimer C, Utz HF, Schön CC, Melchinger AE. Forecasting the accuracy of genomic prediction with different selection targets in the training and prediction set as well as truncation selection. Theor Appl Genet. 2015;128(11):2189–2201.

Schrag TA, Westhues M, Schipprack W, Seifert F, Thiemann A, Scholten S, Melchinger AE. Beyond genomic prediction: combining different types of omics data can improve prediction of hybrid performance in maize. Genetics. 2018;208(4):1373–1385.

Stevens A, Ramirez-Lopez L. An Introduction to the Prospectr Package: R Package Vignette R Package Version 0.2.1; 2020.

Stich B, Benke A, Schmidt M, Urbany C, Shi R, Wirén N. von The maize shoot ionome: its interaction partners, predictive power, and genetic determinants. Plant Cell Environ. 2020;43(9): 2095–2111.

Thavamanikumar S, Dolferus R, Thumma BR. Comparison of genomic selection models to predict flowering time and spike grain number in two hexaploid wheat doubled haploid populations. G3 (Bethesda). 2015;5(10):1991–1998.

Thorwarth P, Ahlemeyer J, Bochard A-M, Krumnacker K, Blümel H, Laubach E, Knöchel N, Cselényi L, Ordon F, Schmid KJ. Genomic prediction ability for yield-related traits in German winter barley elite material. Theor Appl Genet. 2017;130(8):1669–1683.

VSN International. CycdesigN. England (UK): VSN International; 2018. https://vsni.co.uk/software/cycdesign [accessed 2021 Jul 20].

Westhues M, Schrag TA, Heuer C, Thaller G, Utz HF, Schipprack W, Thiemann A, Seifert F, Ehret A, Schlereth A, *et al.* Omics-based hybrid prediction in maize. Theor Appl Genet. 2017;130(9): 1927–1939.

Whittaker JC, Thompson R, Denham MC. Marker-assisted selection using ridge regression. Genet Res. 2000;75(2): 249–252.

Windhausen VS, Atlin GN, Hickey JM, Crossa J, Jannink J-L, Sorrells ME, Raman B, Cairns JE, Tarekegne A, Semagn K, *et al.* Effectiveness of genomic prediction of maize hybrid performance in different breeding populations and environments. G3 (Bethesda). 2012;2(11):1427–1436.

Würschum T. Mapping QTL for agronomic traits in breeding populations. Theor Appl Genet. 2012;125(2):201–210.

Würschum T, Weiß TM, Renner J, Utz HF, Gierl A, Jonczyk R, Römisch-Margl L, Schipprack W, Schön CC, Schrag TA, *et al.* High-resolution association mapping with libraries of immortalized lines from ancestral landraces. Theor Appl Genet. 2021;135: 243–256.

Zhao Y, Gowda M, Liu W, Würschum T, Maurer HP, Longin FH, Ranc N, Reif JC. Accuracy of genomic selection in European maize elite breeding populations. Theor Appl Genet. 2012; 124(4):769–776.

Zhu X, Leiser WL, Hahn V, Würschum T. Training set design in genomic prediction with multiple biparental families. Plant Genome. 2021;14(3):e20124.

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Unraveling the potential of phenomic selection within and among diverse breeding material of maize (*Zea mays* L.)

Supporting information

TABLE S1 Phenotypic raw data and Best Linear Unbiased Estimates of each genotype (BLUEs). Available at figshare: https://doi.org/10.25387/g3.16692409.

TABLE S2 Genotypic raw data of the 50K SNP array. Available at figshare: https://doi.org/10.25387/g3.16692409.

TABLE S3 NIRS raw data between wavelengths of 1 250-2 400 nm, based on seed samples and based on biomass samples of seedlings. Available at figshare: https://doi.org/10.25387/g3.16692409.

TABLE S4 Summary statistics for each group. Groups are abbreviated as WA = Walliser, SM = Satu Mare, SF = Strenzfelder, RT = Sankt Galler Rheintaler, GB = Gelber Badischer, CG = Campan-Galade, EF = elite Flints, and ED = elite Dents. σ_g^2 denotes the genotypic variance, σ_{gxe}^2 the genotype-by-environment interaction variance, and σ_ε^2 the error variance. H^2 is the broad-sense heritability. Traits are abbreviated as follows: anthesis-silking-interval (ASI), EV (early vigor), plant height at harvest (Final PH), grain dry matter content (GDM), grain yield (GYield), phosphorus grain concentration in the kernels (Pconc). All values are based on the three location-year-combinations (Hohenheim 2019 and Hohenheim 2020, Eckartsweier 2020), except for Pconc for which HOH 2020 was not available.

Pop	**Variance component**	**ASI [d]**	**EV [ ]**	**Final PH [cm]**	**GDM [%]**	**GYield [t /ha]**	**Pconc [ppm]**
WA	σ_g^2	12.50	0.68	240	37.20	0.17	34 103
	σ_{gxe}^2	5.51	0.33	47	11.10	0.12	8 888
	H^2	0.85	0.82	0.90	0.89	0.72	0.85
SM	σ_g^2	9.65	1.85	708	21.50	0.60	32 404
	σ_{gxe}^2	4.34	0.51	142	5.29	0.57	14 168
	H^2	0.84	0.90	0.93	0.89	0.73	0.81
SF	σ_g^2	3.06	0.72	196	15.90	0.19	20 704
	σ_{gxe}^2	3.54	0.34	72	8.07	0.24	4 215
	H^2	0.67	0.82	0.85	0.82	0.63	0.82
RT	σ_g^2	1.13	1.10	669	15.70	0.13	64 878
	σ_{gxe}^2	0.52	0.46	79	4.12	0.08	15 050
	H^2	0.69	0.85	0.95	0.88	0.70	0.90
GB	σ_g^2	6.02	1.13	371	7.59	0.45	30 276
	σ_{gxe}^2	3.76	0.51	79	7.65	0.40	1 264
	H^2	0.79	0.84	0.91	0.69	0.74	0.90
CG	σ_g^2	1.44	0.46	423	12.60	0.20	180 186
	σ_{gxe}^2	2.03	0.28	17	37.80	0.07	12 600
	H^2	0.59	0.77	0.97	0.48	0.79	0.96
EF	σ_g^2	1.62	0.26	174	17.50	2.58	34 819
	σ_{gxe}^2	1.00	0.42	38	2.78	0.45	10 284
	H^2	0.71	0.59	0.89	0.91	0.94	0.84
ED	σ_g^2	1.60	0.32	280	34.30	4.94	114 046
	σ_{gxe}^2	1.00	0.16	55	10.80	1.66	9 192
	H^2	0.71	0.77	0.91	0.89	0.90	0.95
overall	σ_ε^2	2.03	0.25	58.50	4.85	0.17	18 435
	H^2	**0.78**	**0.80**	**0.91**	**0.86**	**0.85**	**0.85**

TABLE S5 Phenomic prediction based on NIR spectra of seedling biomass samples (BM; green) compared to maize kernels (Seed; yellow). NIRS was assessed for all 393 available genotypes grown in EWE 2020 for seedling biomass as well as for seed samples. The analysis was performed for the three major groups elite Dents (ED, n = 100), elite Flints (EF, n = 100), and landraces (LR, n = 193). The traits anthesis-silking-interval (ASI), EV (early vigor), plant height at harvest (Final PH), grain dry matter content (GDM), grain yield (GYield), and phosphorus grain concentration in the kernels (Pconc) were predicted by phenomic prediction within and among groups. Predictions within a group were cross-validated with 100 runs.

BM1 Seed	**ASI** [d]	**EV** [ ]	**Final PH** [cm]	**GDM** [%]	**GYield** [t DM/ha]	**P conc** [mg/kg DM]
ED ↻	-0.17	0.51	0.13	0.23	0.00	0.22
	0.20	0.26	-0.19	0.42	0.43	0.70
EF ↻	-0.11	0.27	0.05	-0.02	-0.11	0.24
	0.24	-0.06	-0.06	0.23	0.40	0.56
LR ↻	0.03	0.59	0.31	0.18	0.42	0.18
	0.18	0.20	0.14	0.58	0.26	0.70
ED → EF	-0.07	0.24	-0.13	0.11	0.11	0.26
	0.33	0.12	0.09	0.34	0.45	0.61
ED → LR	0.07	0.41	-0.05	0.04	0.05	-0.05
	0.09	0.22	-0.12	0.53	0.38	0.63
EF → ED	-0.04	0.43	-0.15	0.08	0.08	0.18
	0.24	0.15	0.06	-0.11	0.42	0.71
EF → LR	-0.04	0.38	0.23	-0.03	-0.12	0.05
	0.14	0.01	0.04	0.17	0.34	0.59
LR → ED	0.11	0.43	-0.08	0.07	0.19	-0.04
	0.07	0.35	-0.06	0.66	0.46	0.74
LR → EF	-0.10	0.33	0.21	-0.05	-0.07	0.06
	0.21	0.14	0.08	0.50	0.45	0.65

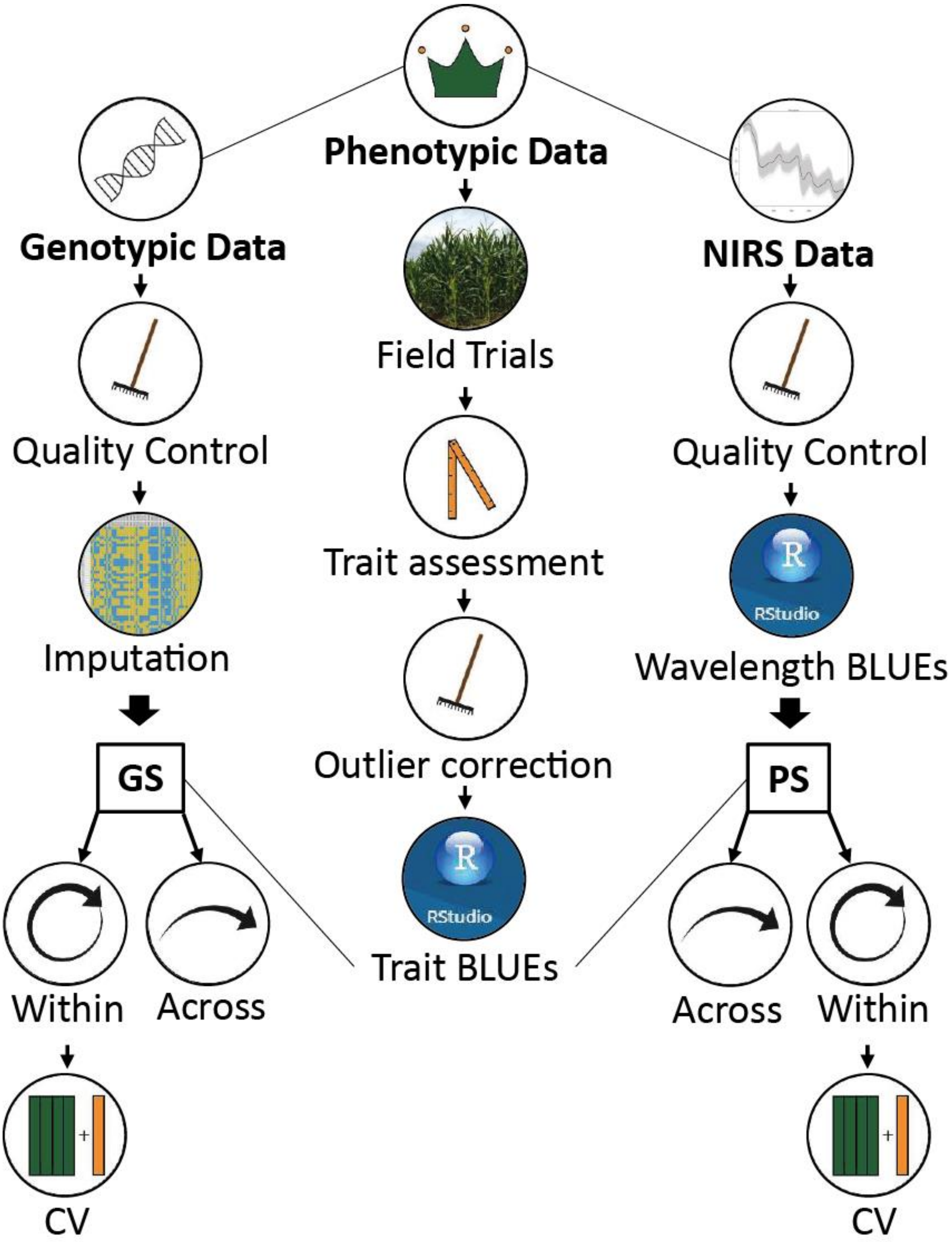

Figure S1 Data processing steps. The different steps of processing the phenotypic (middle), genotypic (left) and NIRS (right) data are depicted. GS stands for genomic prediction, PS for phenomic prediction, BLUEs for best linear unbiased estimates, and CV for cross-validation.

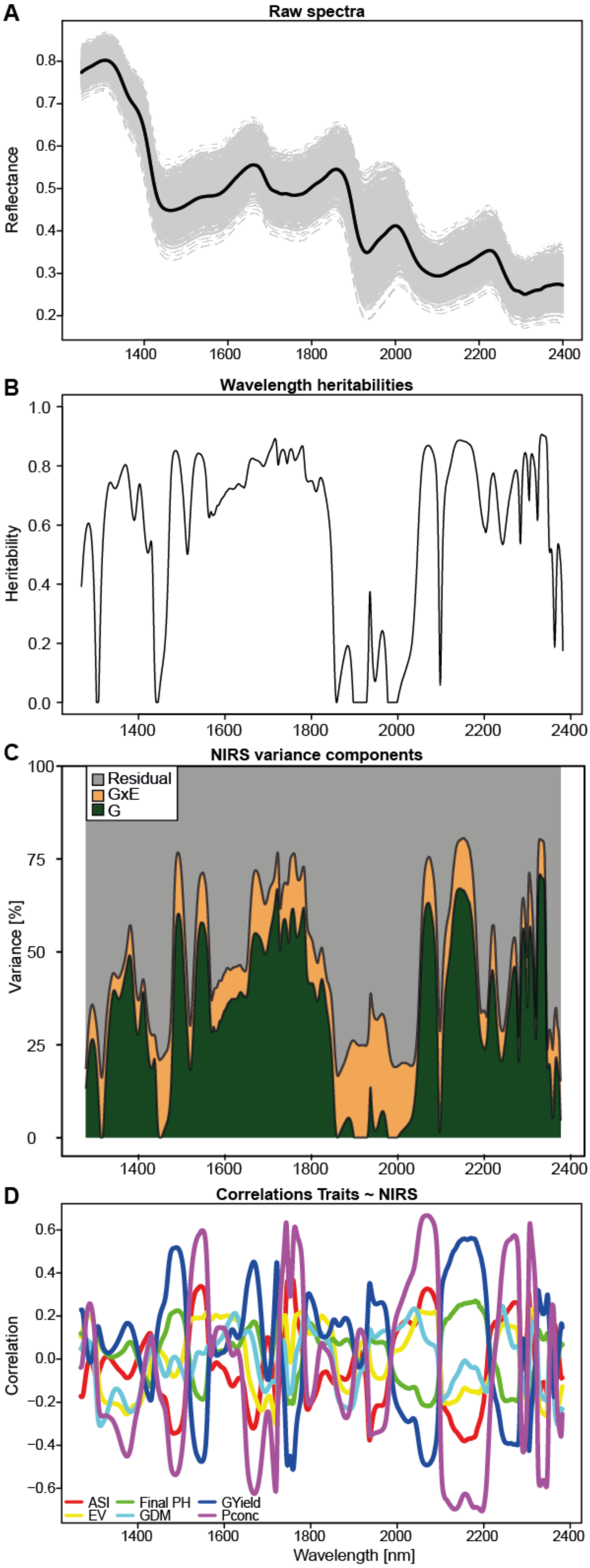
A
Raw spectra
Reflectance
0.2
0.3
0.4
0.5
0.6
0.7
0.8
1400
1600
1800
2000
2200
2400
B
Wavelength heritabilities
Heritability
0.0
0.2
0.4
0.6
0.8
1.0
1400
1600
1800
2000
2200
2400
C
NIRS variance components
Variance [%]
0
25
50
75
100
Residual
GxE
G
1400
1600
1800
2000
2200
2400
D
Correlations Traits ~ NIRS
Correlation
−0.6
−0.4
−0.2
0.0
0.2
0.4
0.6
ASI
Final PH
GYield
EV
GDM
Pconc
1400
1600
1800
2000
2200
2400
Wavelength [nm]

Figure S2 Near infrared spectroscopy data. A. Raw NIR spectra of all 400 genotypes. The black line represents the mean. B. Heritabilities for each wavelength across environments. C. Variance components for each wavelength across environments. G stands for the genetic, GE for the genotype-by-environment interaction, and Residual for the error variance term. D. Correlations for each trait between best linear unbiased estimate of the trait and NIR reflectance values. Traits are abbreviated as ASI (anthesis-silking-interval), EV (early vigor), Final PH (plant height at harvest), GDM (grain dry matter content), GYield (grain yield), and Pconc (phosphorus concentration in kernels).

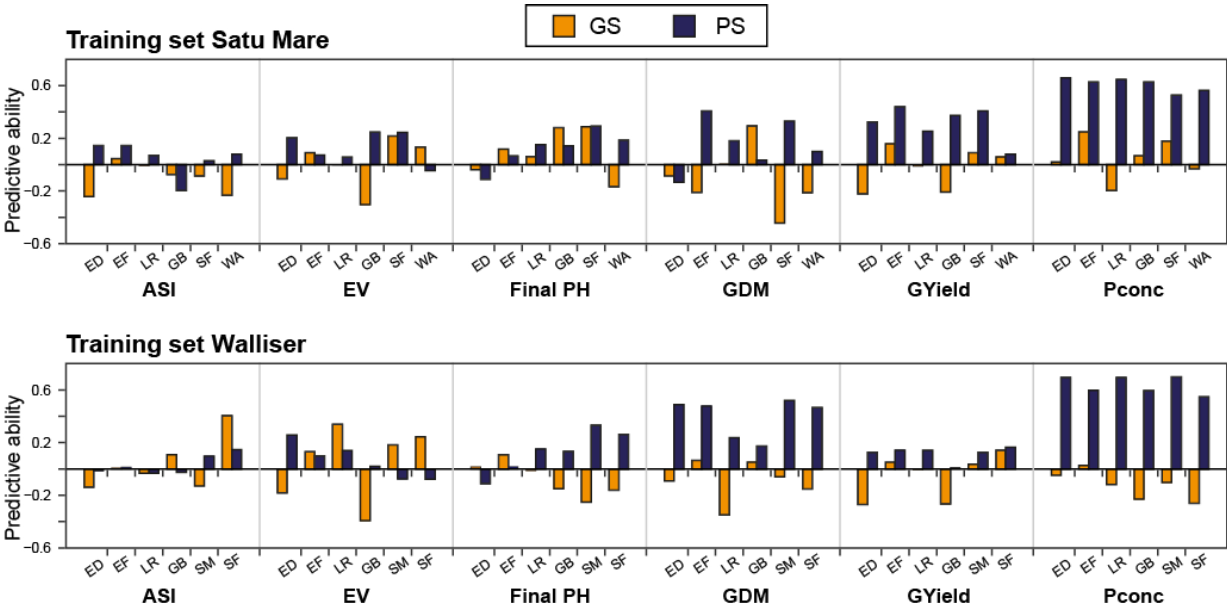

Figure S3 Among-group prediction for Satu Mare and Walliser used as training set. Groups are abbreviated as ED = elite Dents, EF = elite Flints, LR = overall landraces, GB = Gelber Badischer, SM = Satu Mare, SF = Strenzfelder, and WA = Walliser. GS and PS stand for genomic and phenomic prediction, respectively. Traits are denoted as ASI (anthesis-silking-interval), EV (early vigor), Final PH (plant height at harvest), GDM (grain dry matter content), GYield (grain yield), and Pconc (phosphorus concentration in kernels).

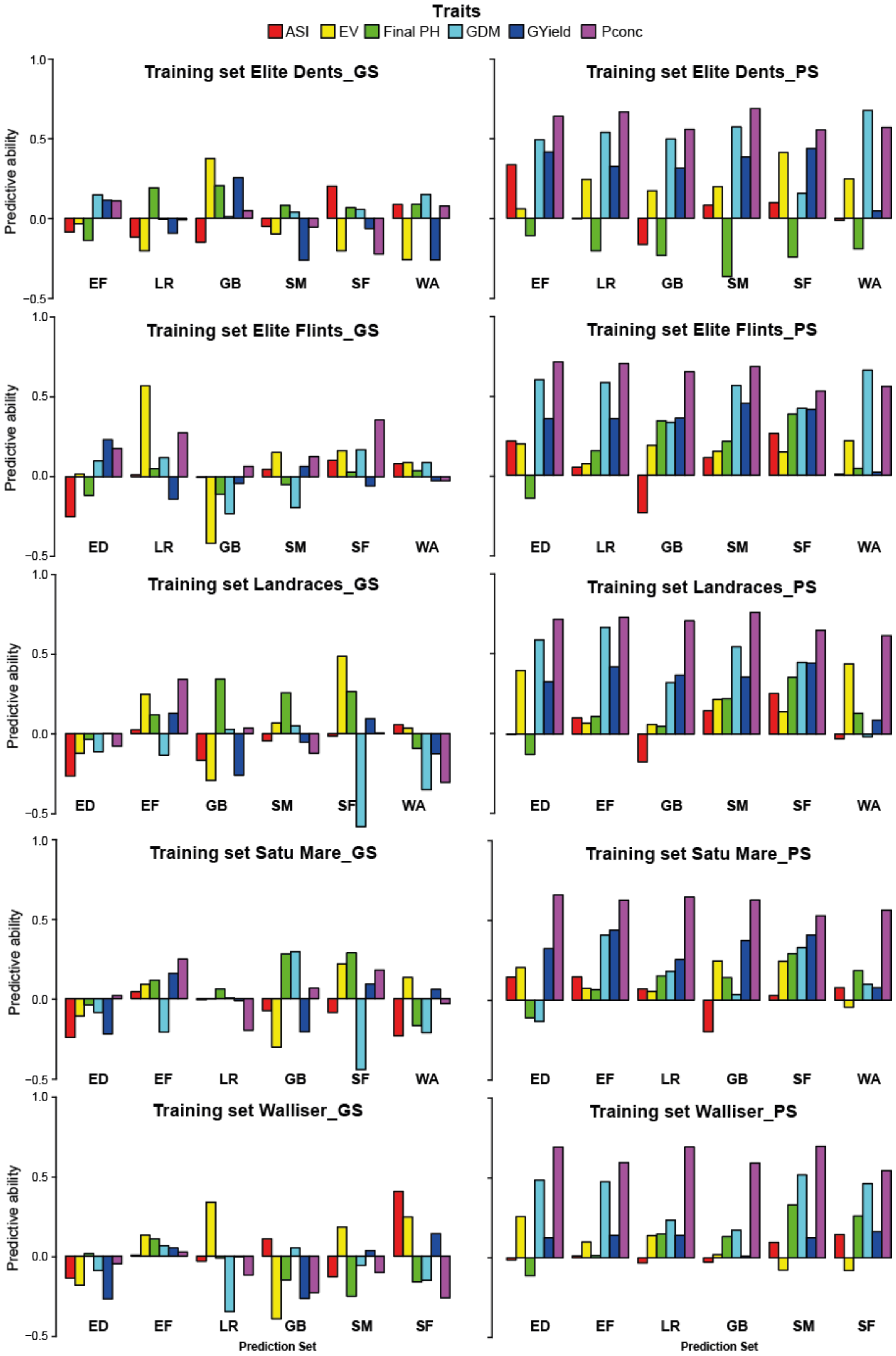
Traits
ASI
EV
Final PH
GDM
GYield
Pconc
Training set Elite Dents_GS
Training set Elite Dents_PS
Training set Elite Flints_GS
Training set Elite Flints_PS
Training set Landraces_GS
Training set Landraces_PS
Training set Satu Mare_GS
Training set Satu Mare_PS
Training set Walliser_GS
Training set Walliser_PS
Predictive ability
1.0
0.5
0.0
−0.5
EF LR GB SM SF WA
ED LR GB SM SF WA
ED EF GB SM SF WA
ED EF LR GB SF WA
ED EF LR GB SM SF
Prediction Set
Prediction Set

Figure S4 Predictive ability of among-group predictions shown by group. Groups are abbreviated as ED = elite Dents, EF = elite Flints, LR = overall landraces, GB = Gelber Badischer, SM = Satu Mare, SF = Strenzfelder, and WA = Walliser. GS (left side) and PS (right side) stand for genomic and phenomic prediction, respectively. Traits are denoted as ASI (anthesis-silking-interval), EV (early vigor), Final PH (plant height at harvest), GDM (grain dry matter content), GYield (grain yield), and Pconc (phosphorus concentration in kernels).

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

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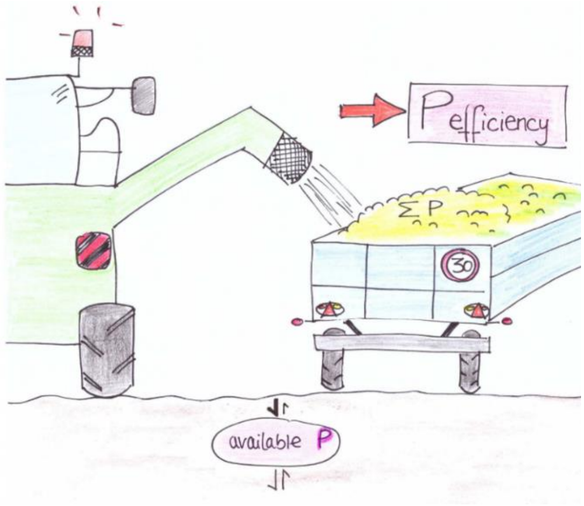

Figure 7 Graphical excerpt of publication 3. The definition of P efficiency as the ratio of harvested crop per unit of P given, largely depends on the harvested farm product as well as on the environment, which eventually determines the available P.

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Short running title: P efficiency of diverse maize lines in well supplied soils

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Abstract

Future farming is required to produce high yields with reduced inputs. Increased fertilizer prices as well as policy goals underline the need to breed for nutrient-efficient varieties. We therefore conducted a multi-environmental field trial comprising 400 maize genotypes, half elite lines, 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: maize – landraces – phosphorus - phosphate efficiency – breeding - genomic selection

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 & Souza Júnior, 2008); a concept which 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-Sahara 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 (*Zea mays* L.) genotypes under – compared to 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 multi-environmental field trials in Germany and assessed them for their biomass yield at seedling stage as well as for biomass and grain yield at harvest, and then analyzed 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 programs focussing on phosphate efficiency.

2. MATERIAL AND METHODS

2.1. Phenotypic Trait Assessment

2.1.1. Germplasm

In this study, we used 400 maize 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 is depicted in Figure S1. It can be seen that the spring of 2019 was extraordinary cold (May 2019: -2.6°C compared to 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 stage 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. In the lab, all samples were analyzed 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 supplementary material 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’ (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) was 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 elite Dents, elite Flints, 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, i.e. the elite Dents, the elite Flints, and the two largest landraces Satu Mare and Walliser separately. We used fivefold cross-validation with 1,000 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: phosphate use efficiency, which itself can be separated into phosphate uptake efficiency and phosphate utilization efficiency. 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 analyzed the summary statistics, variance components and heritabilities across environments (Table 2). When comparing the means, we found that the grain showed a significantly ($P < 0.01$) higher P concentration compared to 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 to 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 0.63 for P content in the seedling to 0.92 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 Satu Mare 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 = 0.46***) with each other. Higher P concentrations of the 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 phosphate use efficiency 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 0.18*** for the seedling and negative correlations of -0.27*** for stover and -0.50*** for grain. We found that P content was highly positively correlated with yield for all tissue types (0.69*** $< r <$ 0.99***), but P content of the grain showed a negative correlation of -0.39 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. Since our panel is composed of different breeding material, it also allows to evaluate the performance of each subgroup (Figure 2, 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 landraces St. Galler Rheintaler, Gelber Badischer and Strenzfelder 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 (elite Flints and all landraces). Moreover, the two subgroups elite Dents and Satu Mare behaved differently than the other groups. While both demonstrated a below-average performance in the early stages, they had a significantly higher phosphate use efficiency of the stover compared to the other subgroups. The elite Dent 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 elite Dent lines except for two elite Flints and one Satu Mare genotype. Taken together, the germplasm as well as the analyzed 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, 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 elite Dents, elite Flints, Satu Mare, and Walliser (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 0.22 and 0.19. 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 Walliser with 0.73 for the P concentration in the seedling, while the lowest mean prediction accuracy of 0.17 was observed for Satu Mare for the P utilization of grain. Furthermore, when comparing the prediction accuracies of each subgroup, the elite Dents showed the highest values across all traits with an average prediction accuracy of 0.53 and the landrace Walliser the lowest with an average prediction accuracy of 0.41. In summary, genomic prediction resulted for most phosphate efficiency measures in moderate to high prediction accuracies.

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.

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 vigor. 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 phosphate use efficiency 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.

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 (Figure 1a, 2, Table S3). We observed a generally superior performance of elite Dent lines in comparison to the Flint subgroups, including a substantially higher grain yield. Except for a significantly lower seedling yield of the elite Dent 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 to Flints and therefore a higher line per se performance under favorable conditions (Moreno‐Gonzalez et al., 1997). Moreover, the Satu Mare landrace lines showed extraordinarily high trait values for phosphate utilization efficiency of the stover. The reason for this is that Satu Mare genotypes frequently showed the phenomenon of barren stalks. Since 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 phosphate utilization efficiency of the stover.

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. Since 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 aluminum 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 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 programs 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, analyzing 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 since 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.

References

Adem, G. D., Ueda, Y., Hayes, P. E., & Wissuwa, M. (2020). Genetic and physiological traits for internal phosphorus utilization efficiency in rice. *PloS One*, *15*(11), e0241842. https://doi.org/10.1371/journal.pone.0241842

Agrometeorology Baden-Württemberg. (2022). *Monthly averages.* Landwirtschaftliches Technologiezentrum Augustenberg. <https://www.wetter-bw.de/Agrarmeteorologie-BW/Wetterdaten/Stationskarte>

Akhtar, S., Osthoff, G., Mashingaidze, K., & Labuschagne, M. (2018). Iron and Zinc in Maize in the Developing World: Deficiency, Availability, and Breeding. *Crop Science*, *58*(6), 2200–2213. https://doi.org/10.2135/cropsci2018.02.0133

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., Sousa, S. M. de, & 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

Bovill, W. D., Huang, C. Y., & McDonald, G. K. (2013). Genetic approaches to enhancing phosphorus-use efficiency (PUE) in crops: challenges and directions. *Crop and Pasture Science*, *64*(3), 179. https://doi.org/10.1071/CP13135

Browning, B. L., Zhou, Y [Ying], & Browning, S. R. (2018). A One-Penny Imputed Genome from Next-Generation Reference Panels. *American Journal of Human Genetics*, *103*(3), 338–348. https://doi.org/10.1016/j.ajhg.2018.07.015

Butler, D. G., Cullis, B. R., Gilmour, A. R., & Gogel, B. J. (2009). *ASReml-R reference manual: ASReml estimates variance components under a general linear mixed model by residual maximum likelihood (REML).* Department of Primary Industries and Fisheries.

Campbell, N. A., & Reece, J. B. (Eds.). (2009). *Biologie* (8.th ed.). Pearson Studium.

Chen, J., Xu, L., Cai, Y [Yilin], & Xu, J. (2009). Identification of QTLs for phosphorus utilization efficiency in maize (*Zea mays* L.) across P levels. *Euphytica*, *167*(2), 245–252. https://doi.org/10.1007/s10681-009-9883-x

Ciampitti, I. A., Camberato, J. J., Murrell, S. T., & Vyn, T. J. (2013). Maize Nutrient Accumulation and Partitioning in Response to Plant Density and Nitrogen Rate: I. Macronutrients. *Agronomy Journal*, *105*(3), 783–795. https://doi.org/10.2134/agronj2012.0467

Ciampitti, I. A., & Vyn, T. J. (2014). Understanding Global and Historical Nutrient Use Efficiencies for Closing Maize Yield Gaps. *Agronomy Journal*, *106*(6), 2107–2117. https://doi.org/10.2134/agronj14.0025

Endelman, J. B. (2011). Ridge Regression and Other Kernels for Genomic Selection with R Package rrBLUP. *The Plant Genome*, *4*(3), 250–255. https://doi.org/10.3835/plantgenome2011.08.0024

Epskamp, S., Cramer, A. O. J., Waldorp, L. J., Schmittmann, V. D., & Borsboom, D. (2012).

qgraph - Network Visualizations of Relationships in Psychometric Data. *Journal of Statistical Software*, *48*(4), 1–18. http://www.jstatsoft.org/v48/i04/

Erel, R., Bérard, A., Capowiez, L., Doussan, C., Arnal, D., Souche, G., Gavaland, A., Fritz, C., Visser, E. J. W., Salvi, S., Le Marié, C., Hund, A., & Hinsinger, P. (2017). Soil type determines how root and rhizosphere traits relate to phosphorus acquisition in field-grown maize genotypes. *Plant and Soil*, *412*(1-2), 115–132. https://doi.org/10.1007/s11104-016-3127-3

European Commission (Ed.). (2020). *Farm to Fork Strategy: For a fair, healthy and environmentally-friendly food system* [#EUGreenDeal]. European Commission. https://ec.europa.eu/food/system/files/2020-05/f2f_action-plan_2020_strategy-info_en.pdf

FAOSTAT. (2019). *Data > Inputs > Fertilizers by nutrient*. http://www.fao.org/faostat/en/#data/RFN

Frank Stephano, M., Geng, Y., Cao, G., Wang, L., Meng, W., & Meiling, Z. (2021). Effect of Silicon Fertilizer and Straw Return on the Maize Yield and Phosphorus Efficiency in Northeast China. *Communications in Soil Science and Plant Analysis*, *52*(2), 116–127. https://doi.org/10.1080/00103624.2020.1854284

Future Committee Agriculture (Ed.). (August 2021). *Zukunft Landwirtschaft. Eine gesamtgesellschaftliche Aufgabe [Future agriculture. An overall social task]: Empfehlungen der Zukunftskommission Landwirtschaft*. Berlin.

Gabelman, W. H., & Gerloff, G. C. (1983). The search for and interpretation of genetic controls that enhance plant growth under deficiency levels of a macronutrient. In M. R. Sarić & B. C. Loughman (Eds.), *Genetic Aspects of Plant Nutrition* (pp. 379–394). Springer Netherlands. https://doi.org/10.1007/978-94-009-6836-3_45

Gamuyao, R., Chin, J. H., Pariasca-Tanaka, J., Pesaresi, P., Catausan, S., Dalid, C., Slamet-Loedin, I., Tecson-Mendoza, E. M., Wissuwa, M., & Heuer, S. (2012). The protein kinase *Pstol1* from traditional rice confers tolerance of phosphorus deficiency. *Nature*, *488*(7412), 535–539. https://doi.org/10.1038/nature11346

Gojon, A., Nussaume, L., Luu, D. T., Murchie, E. H., Baekelandt, A., Rodrigues Saltenis, V. L., Cohan, J.-P., Desnos, T., Inzé, D., Ferguson, J. N., Guiderdonni, E., Krapp, A., Klein Lankhorst, R., Maurel, C., Rouached, H., Parry, M. A. J., Pribil, M., Scharff, L. B., & Nacry, P. (2022). Approaches and determinants to sustainably improve crop production. *Food and Energy Security.* Advance online publication. https://doi.org/10.1002/fes3.369

Hallauer, A., Filho, J. B. M., & Carena, M. J. (2010). *Quantitative genetics in maize breeding* (3rd ed.). *Handbook of plant breeding: Vol. 7290*. Springer.

Huang, M., Liu, X., Zhou, Y [Yao], Summers, R. M., & Zhang, Z. (2019). Blink: A package for the next level of genome-wide association studies with both individuals and markers in the millions. *GigaScience*, *8*(2). https://doi.org/10.1093/gigascience/giy154

Humer, E., & Zebeli, Q. (2015). Phytate in feed ingredients and potentials for improving the utilization of phosphorus in ruminant nutrition. *Animal Feed Science and Technology*, *209*, 1–15. https://doi.org/10.1016/j.anifeedsci.2015.07.028

Hund, A., Ruta, N., & Liedgens, M. (2009). Rooting depth and water use efficiency of tropical maize inbred lines, differing in drought tolerance. *Plant and Soil*, *318*(1-2), 311–325. https://doi.org/10.1007/s11104-008-9843-6

Ibrahim, K., Wang, Q., Le Wang, Zhang, W [Weiwei], Peng, C., & Zhang, S. (2021). Determining the Optimum Level of Soil Olsen Phosphorus and Phosphorus Fertilizer Application for High Phosphorus-Use Efficiency in *Zea mays* L. in Black Soil. *Sustainability*, *13*(11), 5983. https://doi.org/10.3390/su13115983

Jiao, Y., Peluso, P., Shi, J., Liang, T., Stitzer, M. C., Wang, B., Campbell, M. S., Stein, J. C., Wei, X., Chin, C.-S., Guill, K., Regulski, M., Kumari, S., Olson, A., Gent, J., Schneider, K. L., Wolfgruber, T. K., May, M. R., Springer, N. M., . . . Ware, D. (2017). Improved maize reference genome with single-molecule technologies. *Nature*, *546*(7659), 524–527. https://doi.org/10.1038/nature22971

Kassambara, A. (2020). *ggpubr* (Version R package version 0.2.5) [Computer software]. <https://CRAN.R-project.org/package=ggpubr>

Leiser, W. L., Rattunde, H. F. W., Piepho, H.-P., Weltzien, E., Diallo, A., Toure, A., & Haussmann, B. I. (2015). Phosphorous Efficiency and Tolerance Traits for Selection of Sorghum for Performance in Phosphorous-Limited Environments. *Crop Science*, *55*(3), 1152–1162. https://doi.org/10.2135/cropsci2014.05.0392

Leiser, W. L., Rattunde, H. F. W., Weltzien, E., Cisse, N., Abdou, M., Diallo, A., Tourè, A. O., Magalhaes, J. V., & Haussmann, B. I. G. (2014). Two in one sweep: Aluminum tolerance and grain yield in P-limited soils are associated to the same genomic region in West African sorghum. *BMC Plant Biology*, *14*, 206. https://doi.org/10.1186/s12870-014-0206-6

Leiser, W. L., Rattunde, H. F. W., Weltzien, E., & Haussmann, B. I. G. (2014). Phosphorus uptake and use efficiency of diverse West and Central African sorghum genotypes under field conditions in Mali. *Plant and Soil*, *377*(1-2), 383–394. https://doi.org/10.1007/s11104-013-1978-4

Li, D., Chen, Z., Wang, M [Meng], Leiser, W. L., Weiß, T. M., Zhao, Z., Cheng, S., Chen, S., Chen, F., Yuan, L., Würschum, T., & Liu, W. (2021). Dissecting the phenotypic response of maize to low phosphorus soils by field screening of a large diversity panel. *Euphytica*, *217*(1). https://doi.org/10.1007/s10681-020-02727-2

Li, D., Wang, H., Wang, M [Meng], Li, G., Chen, Z., Leiser, W. L., Weiß, T. M., Lu, X., Wang, M [Ming], Chen, S., Chen, F., Yuan, L., Würschum, T., & Liu, W. (2021). Genetic Dissection of Phosphorus Use Efficiency in a Maize Association Population under Two P Levels in the Field. *International Journal of Molecular Sciences*, *22*(17), 9311. https://doi.org/10.3390/ijms22179311

Liao, H., & Yan, X [Xiaolong] (1999). Seed size is closely related to phosphorus use efficiency and photosynthetic phosphorus use efficiency in common bean. *Journal of Plant Nutrition*, *22*(6), 877–888. https://doi.org/10.1080/01904169909365679

Lux, P. E., Fuchs, L., Wiedmaier-Czerny, N., & Frank, J. (2022). Oxidative stability of tocochromanols, carotenoids, and fatty acids in maize (*Zea mays* L.) porridges with varying phytate concentrations during cooking and in vitro digestion. *Food Chemistry*, *378*, 132053. https://doi.org/10.1016/j.foodchem.2022.132053

Mendiburu, F. de. (2020). *agricolae* (Version R package version 1.3-2) [Computer software]. https://CRAN.R-project.org/package=agricolae

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.

Moreno‐Gonzalez, J., Ramos‐Gourcy, F., & Losada, E. (1997). Breeding Potential of European Flint and Earliness‐Selected U.S. Corn Belt Dent Maize Populations. *Crop Science*, *37*(5), 1475–1481. https://doi.org/10.2135/cropsci1997.0011183X003700050010x

Neuweiler, J. E., Maurer, H. P, & Würschum, T. (2021). Genetic architecture of phenotypic indices for simultaneous improvement of protein content and grain yield in triticale (× *triticosecale*). *Plant Breeding*, *140*(2), 232–245. https://doi.org/10.1111/pbr.12894

Nkebiwe, P. M., Weinmann, M., Bar-Tal, A., & Müller, T. (2016). Fertilizer placement to improve crop nutrient acquisition and yield: A review and meta-analysis. *Field Crops Research*, *196*, 389–401. https://doi.org/10.1016/j.fcr.2016.07.018

Parentoni, S. N., & Souza Júnior, C. L. de (2008). Phosphorus acquisition and internal utilization efficiency in tropical maize genotypes. *Pesq. Agropec. Bras., Brasília*, *43*(7), 893–901.

Rebourg, C., Chastanet, M., Gouesnard, B., Welcker, C., Dubreuil, P., & Charcosset, A (2003). Maize introduction into Europe: The history reviewed in the light of molecular data. *TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik*, *106*(5), 895–903. https://doi.org/10.1007/s00122-002-1140-9

Redel, Y., Kunz, E., Hartmann, T. E., Müller, T., & Bol, R. (2021). Long-Term Compost Application and the Impact of Soil P Legacy on the Enhancement of Early Maize Growth. *Journal of Soil Science and Plant Nutrition*, *21*(1), 873–881. https://doi.org/10.1007/s42729-020-00407-7

Revilla, P., Rodríguez, V. M., Ordás, A., Rincent, R., Charcosset, A , Giauffret, C., Melchinger, A. E., Schön, C.-C., Bauer, E., Altmann, T., Brunel, D., Moreno-González, J., Campo, L., Ouzunova, M., Laborde, J., Álvarez, Á., Ruíz de Galarreta, J. I., & Malvar, R. A. (2014). Cold Tolerance in Two Large Maize Inbred Panels Adapted to European Climates. *Crop Science*, *54*(5), 1981–1991. https://doi.org/10.2135/cropsci2013.11.0733

Rodehutscord, M., Rückert, C., Maurer, H. P, Schenkel, H., Schipprack, W., Bach Knudsen, K. E., Schollenberger, M., Laux, M., Eklund, M., Siegert, W., & Mosenthin, R. (2016). Variation in chemical composition and physical characteristics of cereal grains from different genotypes. *Archives of Animal Nutrition*, *70*(2), 87–107. https://doi.org/10.1080/1745039X.2015.1133111

Rose, T. J., Mori, A., Julia, C. C., & Wissuwa, M. (2016). Screening for internal phosphorus utilisation efficiency: comparison of genotypes at equal shoot P content is critical. *Plant and Soil*, *401*(1-2), 79–91. https://doi.org/10.1007/s11104-015-2565-7

Rose, T. J., & Wissuwa, M. (2012). *Rethinking Internal Phosphorus Utilization Efficiency: A*

New Approach is Needed to Improve PUE in Grain Crops. Advances in Agronomy: Vol. 116. Elsevier Inc. Academic Press.

RStudio Team. (2020). *RStudio: Integrated Development Environment for R* (Version 3.5.3) [Computer software]. RStudio, PBC. Boston, MA USA. <http://www.rstudio.com/>

Strigens, A., Freitag, N. M., Gilbert, X., Grieder, C., Riedelsheimer, C., Schrag, T. A., Messmer, R., & Melchinger, A. E. (2013). Association mapping for chilling tolerance in elite flint and dent maize inbred lines evaluated in growth chamber and field experiments. *Plant, Cell & Environment*, *36*(10), 1871–1887. https://doi.org/10.1111/pce.12096

Tiedemann, F. (2020). *gghalves: Compose Half-Half Plots Using Your Favourite Geoms.* (Version R package version 0.1.1.) [Computer software]. <https://CRAN.R-project.org/package=gghalves>

Turner, S. D. (2018). qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. *Journal of Open Source Software*, *3*(25), 731. https://doi.org/10.21105/joss.00731

VSN International. (2018). *CycdesigN* (Version 6.0) [Computer software]. VSN International. Hemel Hempstead, England UK. <https://www.vsni.co.uk>

Vu, V. Q. (2011). *ggbiplot: A ggplot2 based biplot.* (Version R package version 0.55) [Computer software]. <http://github.com/vqv/ggbiplot>

Wang, J., & Zhang, Z. (2021). Gapit Version 3: Boosting Power and Accuracy for Genomic Association and Prediction. *Genomics, Proteomics & Bioinformatics.* Advance online publication. https://doi.org/10.1016/j.gpb.2021.08.005

Weiß, T. M., Leiser, W. L., Reineke, A.-J., Li, D., Liu, W., Hahn, V., & Würschum, T. (2021). Optimizing the P balance: How do modern maize hybrids react to different starter fertilizers? *PloS One*, *16*(4), e0250496. https://doi.org/10.1371/journal.pone.0250496

Weiß, T. M., Zhu, X., Leiser, W. L., Li, D., Liu, W., Schipprack, W., Melchinger, A. E., Hahn, V., & Würschum, T. (2022). Unraveling the potential of phenomic selection within and among diverse breeding material of maize (*Zea mays* L.). *G3 Genes|Genomes|Genetics*, Article jkab445. Advance online publication. https://doi.org/10.1093/g3journal/jkab445

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., . . . Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Wickham, H., Hester, J., & Chang, W. (2021). *devtools: Tools to Make Developing R Packages Easier.* (Version R package version 2.4.2) [Computer software]. <https://CRAN.R-project.org/package=devtools>

Wissuwa, M., & Ae, N. (2001). Genotypic variation for tolerance to phosphorus defciency in rice and the potential for its exploitation in rice improvement. *Plant Breeding*(120), 43–48.

Wissuwa, M., Yano, M., & Ae, N. (1998). Mapping of QTLs for phosphorus-deficiency tolerance in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, *97*(5-6), 777–783. https://doi.org/10.1007/s001220050955

Withers, P. J. A., Vadas, P. A., Uusitalo, R., Forber, K. J., Hart, M., Foy, R. H., Delgado, A., Dougherty, W., Lilja, H., Burkitt, L. L., Rubæk, G. H., Pote, D., Barlow, K., Rothwell, S., & Owens, P. R. (2019). A Global Perspective on Integrated Strategies to Manage Soil Phosphorus Status for Eutrophication Control without Limiting Land Productivity. *Journal of Environmental Quality*, *48*(5), 1234–1246. https://doi.org/10.2134/jeq2019.03.0131

Würschum, T., Weiß, T. M., Renner, J., Friedrich Utz, H., Gierl, A., Jonczyk, R., Römisch-Margl, L., Schipprack, W., Schön, C.-C., Schrag, T. A., Leiser, W. L., & Melchinger, A. E. (2022). High-resolution association mapping with libraries of immortalized lines from ancestral landraces. *TAG. Theoretical and Applied Genetics*(135), 243–256. https://doi.org/10.1007/s00122-021-03963-3

Xin, X., Qin, S., Zhang, J., Zhu, A., Yang, W., & Zhang, X. (2017). Yield, phosphorus use efficiency and balance response to substituting long-term chemical fertilizer use with organic manure in a wheat-maize system. *Field Crops Research*, *208*, 27–33. https://doi.org/10.1016/j.fcr.2017.03.011

Yan, X [Xiaojun], Chen, X [Xiaohui], Ma, C., Cai, Y [Yuanyang], Cui, Z., Chen, X [Xinping], Wu, L., & Zhang, F. (2021). What are the key factors affecting maize yield response to and agronomic efficiency of phosphorus fertilizer in China? *Field Crops Research*, *270*, 108221. https://doi.org/10.1016/j.fcr.2021.108221

Zhang, W [Wei], Zhang, W [Wushuai], Wang, X., Liu, D., Zou, C., & Chen, X [Xinping] (2021). Quantitative evaluation of the grain zinc in cereal crops caused by phosphorus fertilization. A meta-analysis. *Agronomy for Sustainable Development*, *41*(1). https://doi.org/10.1007/s13593-020-00661-0

Zhao, Y., Gowda, M., Liu, W., Würschum, T., Maurer, H. P, Longin, F. H., Ranc, N., & Reif, J. C. (2012). Accuracy of genomic selection in European maize elite breeding populations. *Theoretical and Applied Genetics*, *124*(4), 769–776. https://doi.org/10.1007/s00122-011-1745-y

TABLES

Table 1 Different phosphate efficiency measures reviewed in literature. 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, biomass). Abbreviations, which were eventually used in this sutdy 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.

Category	Name	Abbr.	Calculation	Crop	Reference
PUE	P-use efficiency	**SeY** **StY** **GY**	$PUE = Yield$ $PUE = SeedlingYield$ $PUE = StoverYield$ $PUE = GrainYield$	Maize; Rice	Adem et al., 2020; Azevedo et al., 2015
PUE	Agronomic efficiency; P-use efficiency; Partial factor productivity of P	agroPUE	$PUE = PUpE * PUtE = \frac{Yield}{P_{supplied}}$	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**	$PUpE = GY * Pconc_{Grain}$	Maize; Rice; Sorghum	Azevedo et al., 2015; Ciampitti & Vyn, 2014; Leiser et al., 2015; Li, Chen, et al., 2021; Rose et al., 2016; Wissuwa & Ae, 2001
PUpE	P content; Per-plant nutrient uptake	**Pcont Se** **Pcont St**	$PUpE = \frac{Yield*Pconc}{Plant\ density}$	Maize	Ciampitti & Vyn, 2014
PUtE	Physiological efficiency; P utilization efficiency; P utilization grain	**PUtE G**	$PUtE = \frac{GY}{Pcont_{Biomass}}$	Maize; Sorghum	Ciampitti & Vyn, 2014; Leiser et al., 2015; Moll et al., 1982; Parentoni & Souza Júnior, 2008

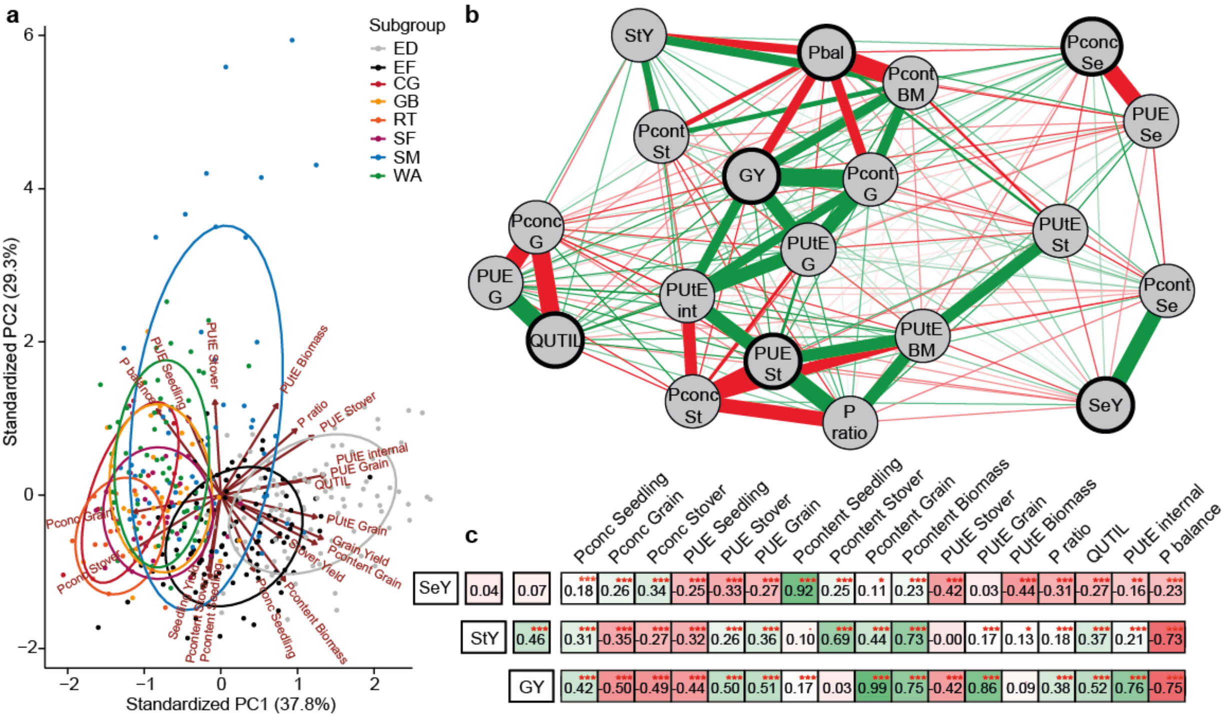
PUtE	P utilization stover	**PUtE St**	$PUtE = \frac{StY}{Pcont_{Biomass}}$	Sorghum; Rice	Adem et al., 2020; Leiser et al., 2015
PUtE	P utilization biomass	**PutE BM**	$PUtE = \frac{GY+StY}{Pcont_{Biomass}}$	Tomato; Bean; Sorghum	Gabelman & Gerloff, 1983; Leiser et al., 2015
PUtE	P ratio	**P ratio**	$PUtE = \frac{Pconc_{Grain}}{Pconc_{Stover}}$	Cereals	Zhang et al., 2021
PUtE	P harvest index	PHI	$PHI = \frac{Pcont_{Grain}}{Pcont_{Biomass}}$	Maize; Sorghum	Leiser et al., 2015; Parentoni & Souza Júnior, 2008
PUtE	P quotient of utilizaion	**QUTIL**	$PUtE = \frac{GY}{Pcont_{Grain}}$	Maize	Parentoni & Souza Júnior, 2008
PUtE	P internal utilization efficiency	PUTIL	$PUTIL = QUTIL * PHI$	Maize	Parentoni & Souza Júnior, 2008
PUtE	P internal utilization efficiency	**PUtE int**	$PUtE = \frac{BMY}{Pcont_{Stover}}$	Bean; Rice	Ciampitti et al., 2013; Liao & Yan, 1999; Rose et al., 2016
Pbal	P balance	**Pbal**	$Pbal = P_{supplied} - P_{plant\ uptake}$	Maize	Redel et al., 2021

Table 2 Summary statistics for all phosphate efficiency measures. 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), 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.

Variable	**Unit**	**Mean**	σ_g^2	$\sigma_{g\times e}^2$	σ_e^2	σ_g^2:$\sigma_{g\times e}^2$	H^2	**GCV**
SeY♣	[g/4 plants]	6.42	1.91	1.47	1.43	1.30	0.72	0.21
Pconc Seedling♣	[ppm]	3176.38	96799.02	63288.92	117411.66	1.53	0.70	0.10
StY	[g/plant]	41.96	183.31	16.69	82.83	10.98	0.86	0.32
Pconc Stover	[ppm]	2161.83	265117.83	30374.25	88047.95	8.73	0.88	0.24
GY♣	[t/ha]	1.84	1.35	0.58	0.16	2.31	0.86	0.63
Pconc Grain♣	[ppm]	3181.55	52688.89	11733.83	23984.76	4.49	0.87	0.07
PUE Se♣	[kg/g P]	0.34	1.30E-03	8.94E-04	1.59E-03	1.46	0.70	0.11
PUE St	[kg/g P]	0.53	1.88E-02	6.75E-03	6.78E-03	2.78	0.79	0.26
PUE G♣	[kg/g P]	0.32	5.48E-04	1.37E-04	2.34E-04	4.00	0.87	0.07
Pcont Se♣	[g/plant]	0.01	1.32E-06	1.58E-06	1.50E-06	0.84	0.63	0.21
Pcont St	[g/plant]	0.09	6.83E-04	2.52E-04	5.54E-04	2.71	0.72	0.30
Pcont G♣	[kg/ha]	5.58	11.43	4.23	1.77	2.70	0.87	0.61
Pcont BM	[kg/ha]	12.41	15.20	2.39	5.04	6.35	0.86	0.31
PUtE St	[g/mg P]	0.32	8.60E-03	9.76E-04	2.12E-03	8.81	0.89	0.29
PUtE G	[kg/g P]	0.11	2.93E-03	6.96E-04	6.97E-04	4.21	0.85	0.50
PUtE BM	[kg/g P]	0.42	6.97E-03	4.03E-04	1.50E-03	17.28	0.92	0.20

P ratio	[%]	172.17	1580.12	486.12	797.76	3.25	0.78	0.23
QUTIL♣	[ ]	318.42	557.6	145.44	230.17	3.83	0.87	0.07
PUtE Int	[ ]	776.50	78312.58	43588.6	32097.38	1.80	0.72	0.36
Pbal	[kg/ha]	-12.41	15.20	2.39	5.04	6.35	0.86	-0.31

FIGURES



			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, i.e. 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), 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 < 0.01), ‘***’ (p-value < 0.001).

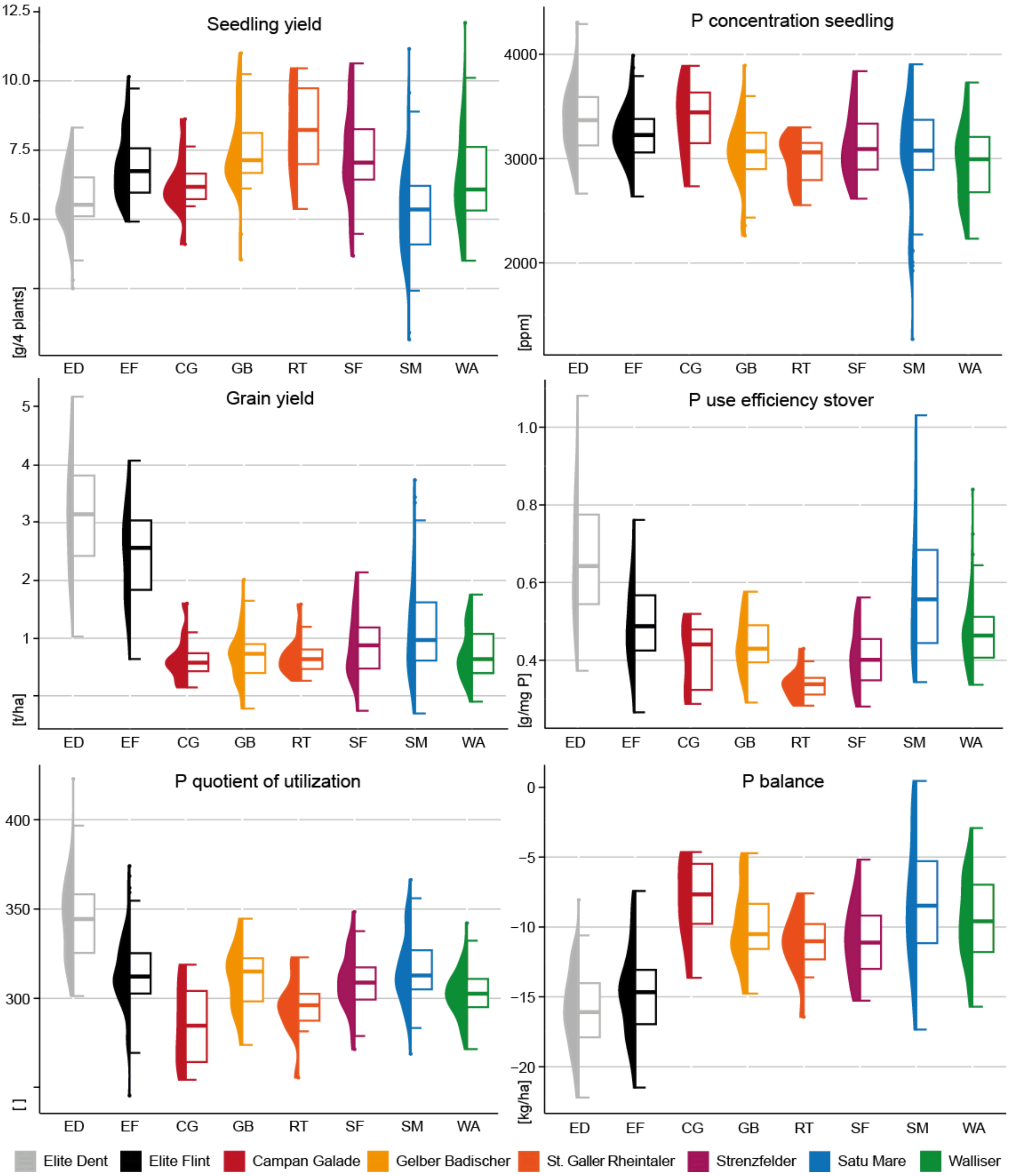
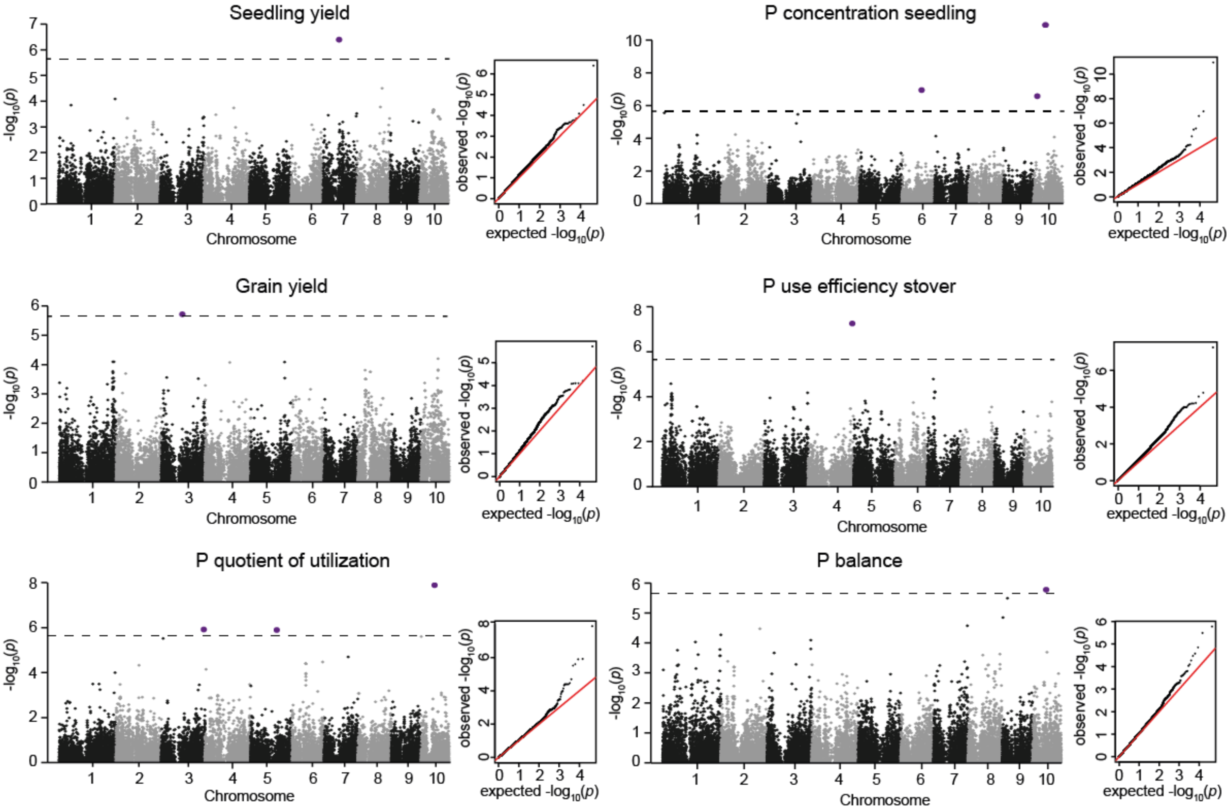


Figure 2 Phenotypic distribution of six exemplary traits representing the phosphate efficiency measures, shown for the different subgroups.


Seedling yield

P concentration seedling

Grain yield

P use efficiency stover

P quotient of utilization

P balance

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, significant SNPs are highlighted in purple.

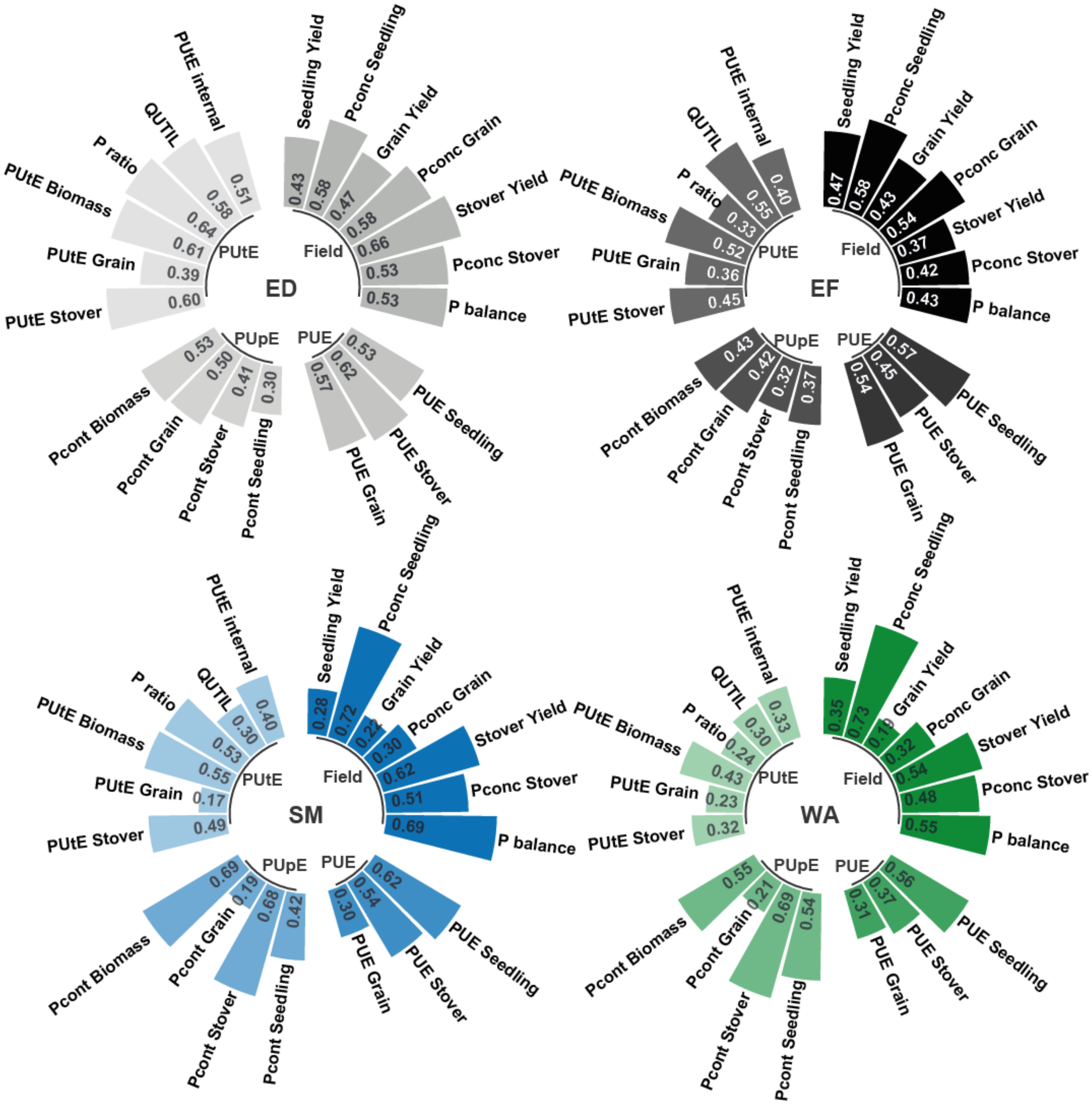


Figure 4 Genomic prediction results for the 20 phosphate use efficiency components and measures, split up into the category 'Field', phosphate use efficiency (PUE), phosphate uptake efficiency (PUpE), and phosphate utilization efficiency (PUtE). Results are given as prediction accuracy obtained with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

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, T.W. and W.L.L.; Methodology, T.W. and W.L.L.; Formal Analysis, T.M.W. and D.L.; Investigation, T.M.W.; Data Curation, T.M.W.; Writing – Original Draft Preparation, T.M.W. and T.W.; Writing – Review & Editing, T.M.W., W.L.L., D.L., S.R., W.L., V.H., T.W.; Visualization, T.M.W. and T.W.; Supervision, T.W., W.L.L., W.L.; Funding Acquisition, T.W.

SUPPLEMENTARY MATERIALS

Table S1 Location characterization including maize cultivation parameters and soil properties of the growing seasons 2019 and 2020.

Parameters [Unit] / Enviro-ments	Altitude [m ASL]	Ø Temperature* [°C]	Ø Precipitation* [mm]	Sowing [Date]	Harvest [Date]	Soil type	pH	Mg [mg/100g soil]	P_2O_5** [mg/100g soil]	P** [mg/100g soil]	Classification P availability***
Heidfeldhof 2019	402	10.6	857	Apr 30th	Oct 14th - 24th	Silty loam	6.79	17.5	19.5	8.5	D
Heidfeldhof 2020	402	10.9	667	Apr 20th	Sep 16th – Oct 13th	Silty loam	6.95	11	14.4	6.3	D
Eckartsweie 2020	142	12.2	659	Apr 15th	Aug 31st - Sep 16th	Clayey loam	5.66	19.6	9.9	4.3	C

* Data retrieved from <www.wetter-bw.de>

** Calcium Acetate lactate (CAL) method and photometric determination of available soil P

*** According to VDLUFA-P-content-classes (A = very low, E = very high) defined by the Association of German Agricultural Analytic and Research Institutes (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten).

Table S2 Field raw data of measured and calculated phosphate efficiency measures.

See separate Excel file for Table S2.

Table S3 Population-specific variance components of the investigated phosphate efficiency measures. 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. Traits are abbreviated 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), 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), grain (PUtE G), biomass (PUtE BM), P ratio, P quotient of utilization (QUTIL), P internal utilization efficiency (PUtE int), and P balance (Pbal). If nothing is indicated, the population-specific values are based on the 2020 field data only, three location-year-combinations (Hohenheim 2019 and Hohenheim 2020, Eckartsweier 2020) are marked with ♣. Different superscript letters indicate significant (*p*-value < 0.05) differences between the eight subgroup means.

Subgroup	Variable	SeY♣ [g/4 plants]	Pconc Seedling♣ [ppm]	StY [g/plant]	Pconc Stover [ppm]	GY♣ [t/ha]	Pconc Grain♣ [ppm]	PUE Se♣ [kg/g P]	PUE St [kg/g P]	PUE G♣ [kg/g P]	Pcont Se♣ [g/plant]	Pcont St [g/plant]	Pcont G♣ [kg/ha]	Pcont BM [kg/ha]	PUtE St [g/mg P]	PUtE G [kg/g P]	PUtE BM [kg/g P]	P ratio [%]	QUTIL♣ [ ]	PUtE Int [ ]	Pbal [kg/ha]
WA	***mean***	6.42^{bc}	2957.03^{b}	37.15^{bc}	2321.89^{b}	0.74^{d}	3324.76^{b}	0.37^{a}	0.47^{cd}	0.30^{cd}	0.01^{bc}	0.08^{a}	2.36^{c}	9.45^{b}	0.36^{b}	0.07^{bc}	0.42^{b}	162.75^{b}	302.65^{cd}	581.04^{c}	-9.45^{a}
	σ_g^2	2.11	1.51E+05	92.55	9.85E+04	0.14	1.85E+04	2.41E-03	4.36E-03	1.62E-04	1.99E-06	6.50E-04	1.75	6.41	3.69E-03	1.07E-03	2.62E-03	394.00	167.00	14939.91	6.41
	$\sigma_{g\times e}^2$	1.62	4.11E+04	23.68	3.76E+04	0.11	1.79E+04	1.48E-03	5.98E-03	1.27E-04	1.65E-06	2.50E-04	0.94	0.88	1.04E-03	2.09E-04	1.27E-03	683.01	125.42	10698.70	0.88
	H^2	0.73	0.82	0.74	0.71	0.69	0.65	0.76	0.49	0.66	0.71	0.71	0.74	0.79	0.78	0.79	0.72	0.42	0.68	0.54	0.79
SM	***mean***	5.28^{d}	3023.51^{ab}	33.30^{c}	1938.76^{c}	1.20^{c}	3193.47^{c}	0.36^{ab}	0.58^{b}	0.32^{b}	0.00^{d}	0.07^{b}	3.70^{c}	8.29^{b}	0.41^{a}	0.09^{b}	0.49^{a}	194.74^{a}	316.23^{b}	764.77^{b}	-8.29^{a}
	σ_g^2	3.17	2.13E+05	206.61	2.17E+05	0.66	3.19E+04	4.16E-03	2.48E-02	3.21E-04	2.82E-06	1.10E-03	6.24	16.96	3.06E-02	1.92E-03	2.48E-02	3281.66	325.73	55532.39	16.96
	$\sigma_{g\times e}^2$	3.58	2.48E+05	0.54	3.57E+04	0.60	1.43E+04	3.54E-03	8.32E-03	1.09E-04	4.32E-06	2.59E-10	4.99	0.00	9.30E-04	7.47E-04	3.76E-04	194.62	118.13	26481.72	0.00
	H^2	0.69	0.68	0.91	0.84	0.74	0.78	0.74	0.81	0.81	0.63	0.89	0.76	0.93	0.97	0.78	0.98	0.92	0.81	0.73	0.93
SF	***mean***	7.34^{ab}	3136.91^{ab}	38.13^{bc}	2615.62^{b}	0.89^{cd}	3263.22^{bc}	0.35^{ab}	0.41^{de}	0.31^{bc}	0.01^{ab}	0.10^{a}	2.80^{c}	11.00^{b}	0.31^{bc}	0.06^{bc}	0.37^{bc}	137.04^{bc}	309.43^{bc}	499.47^{c}	-11^{a}
	σ_g^2	2.94	7.56E+04	35.14	1.91E+05	0.18	2.12E+04	7.17E-04	3.66E-03	1.85E-04	1.36E-06	1.77E-04	1.68	3.19	1.90E-03	8.60E-04	2.46E-03	396.53	188.29	11453.43	3.19
	$\sigma_{g\times e}^2$	1.66	3.13E+04	35.57	2.70E+04	0.24	1.29E+04	4.55E-04	5.19E-04	8.15E-05	1.78E-06	5.55E-04	2.23	3.21	4.20E-04	4.82E-04	1.48E-10	0.00	93.72	0.01	3.21

	H^2	0.79	0.72	0.48	0.84	0.63	0.72	0.63	0.66	0.74	0.62	0.30	0.62	0.53	0.72	0.68	0.87	0.67	0.73	0.60	0.53
RT	***mean***	8.20^{a}	2985.20^{ab}	33.35^{bc}	3072.53^{a}	0.70^{d}	3410.13^{ab}	0.36^{ab}	0.34^{e}	0.30^{cd}	0.01^{a}	0.10^{a}	2.34^{c}	11.21^{b}	0.27c^{d}	0.05^{bc}	0.32^{c}	120.51^{c}	295.81^{cd}	403.76^{c}	-11.21^{a}
	σ_g^2	4.72	7.17E+04	27.05	9.66E+04	0.10	6.06E+04	1.14E-03	6.62E-10	4.46E-04	2.36E-06	3.01E-05	0.98	2.54	1.03E-03	1.38E-04	7.54E-04	61.86	415.55	0.05	2.54
	$\sigma_{g\times e}^2$	2.41	1.18E-02	7.03	1.84E+03	0.07	8.93E+03	2.57E-04	7.17E-04	3.21E-05	1.88E-06	5.20E-04	0.62	1.06	3.32E-09	1.24E-04	2.33E-09	0.00	58.18	0.00	1.06
	H^2	0.82	0.79	0.53	0.81	0.66	0.90	0.76	0.00	0.90	0.73	0.07	0.66	0.59	0.67	0.37	0.67	0.24	0.88	0.00	0.59
GB	***mean***	7.45^{a}	3067.83^{ab}	39.56^{bc}	2432.61^{b}	0.71^{d}	3249.47^{bc}	0.35^{ab}	0.44^{cde}	0.31^{bc}	0.01^{ab}	0.09^{a}	2.22^{c}	9.83^{b}	0.36^{bc}	0.05^{c}	0.41^{b}	148.48^{bc}	311.54^{bc}	513.09^{c}	-9.83^{a}
	σ_g^2	2.67	9.36E+04	94.86	1.27E+05	0.15	2.36E+04	1.42E-03	3.32E-03	2.08E-04	1.52E-06	6.24E-04	1.58	4.11	2.81E-03	7.30E-04	2.51E-03	448.87	180.99	5224.85	4.11
	$\sigma_{g\times e}^2$	1.56	6.96E+04	1.43	5.95E+03	0.13	8.51E+03	1.01E-03	6.62E-10	1.05E-04	1.76E-06	1.84E-04	1.26	3.08	2.00E-03	2.92E-04	7.97E-04	0.00	119.04	0.00	3.08
	H^2	0.78	0.69	0.82	0.84	0.68	0.78	0.70	0.67	0.74	0.65	0.73	0.69	0.60	0.65	0.70	0.77	0.70	0.70	0.41	0.60
CG	***mean***	6.26^{bcd}	3378.56^{a}	27.07^{c}	2618.35^{ab}	0.65^{d}	3542.86^{a}	0.33^{bc}	0.41^{cde}	0.29^{d}	0.01^{abc}	0.07^{ab}	2.30^{c}	8.11^{b}	0.30^{bcd}	0.07^{bc}	0.37^{bc}	153.88^{bc}	285.81^{d}	509.28^{c}	-8.11^{a}
	σ_g^2	0.85	5.57E+04	46.21	2.99E+05	0.16	1.57E+05	3.66E-04	5.32E-03	1.06E-03	6.84E-07	3.58E-04	1.96	6.57	4.87E-03	8.15E-04	3.77E-03	917.78	1090.32	7256.47	6.57
	$\sigma_{g\times e}^2$	0.23	1.47E+05	0.00	4.84E+04	0.06	6.94E+03	1.24E-03	1.55E-03	4.18E-11	2.13E-06	9.21E-11	0.62	0.22	2.65E-09	2.36E-04	4.69E-04	117.20	1.75	2656.87	0.22

	H^2	0.73	0.45	0.69	0.87	0.77	0.96	0.35	0.69	0.96	0.42	0.72	0.80	0.83	0.90	0.74	0.86	0.78	0.97	0.45	0.83
EF	***mean***	6.93^{ab}	3239.03^{a}	39.68^{b}	2231.26^{b}	2.46^{b}	3211.18^{c}	0.33^{bc}	0.5^{c}	0.31^{b}	0.01^{ab}	0.09^{a}	7.78^{b}	14.67^{a}	0.24^{d}	0.15^{a}	0.39^{bc}	162.67^{b}	314.4^{b}	853.91^{b}	-14.67
	σ_g^2	0.94	3.40E+04	84.57	1.59E+05	2.82	3.95E+04	3.40E-04	7.44E-03	3.60E-04	5.96E-07	6.91E-04	26.99	6.94	2.14E-03	1.35E-03	1.71E-03	755.41	361.10	41688.24	6.94
	$\sigma_{g\times e}^2$	1.15	2.28E+04	4.00	3.13E+04	0.46	1.16E+04	5.32E-05	2.85E-03	1.06E-04	9.87E-07	1.36E-04	3.85	1.35	2.10E-10	2.51E-04	1.38E-05	202.78	117.10	27685.46	1.35
	H^2	0.60	0.56	0.79	0.81	0.94	0.83	0.55	0.71	0.83	0.51	0.77	0.94	0.78	0.81	0.82	0.82	0.72	0.82	0.66	0.78
ED	***mean***	5.69^{cd}	3375.28^{a}	56.45^{a}	1715.70^{c}	3.15^{a}	2943.15^{d}	0.32^{c}	0.66^{a}	0.34^{a}	0.01^{cd}	0.09^{a}	8.99^{a}	15.95^{a}	0.31^{bc}	0.15^{a}	0.47^{a}	202.63^{a}	344.55^{a}	1069.6^{a}	-15.95
	σ_g^2	1.24	8.94E+04	131.37	1.24E+05	5.24	1.13E+05	6.54E-04	1.41E-02	1.43E-03	7.52E-07	3.94E-04	38.44	3.82	2.44E-03	9.96E-04	3.03E-03	1430.46	1493.37	35700.02	3.82
	$\sigma_{g\times e}^2$	0.63	4.26E+04	40.45	3.20E+04	1.72	9.20E+03	2.92E-04	1.84E-02	2.40E-04	7.83E-07	4.58E-04	10.22	5.37	1.99E-03	1.83E-03	5.83E-04	1221.92	248.80	153485.98	5.37
	H^2	0.74	0.73	0.76	0.77	0.90	0.94	0.64	0.56	0.92	0.60	0.52	0.91	0.49	0.62	0.48	0.82	0.64	0.92	0.30	0.49

Table S4 Markers reaching the Bonferroni-corrected significance threshold of 5.65 in the genome-wide association study. Frequency indicates how often this SNP was identified as a significant marker for the 20 investigated phosphate efficiency measures.

Trait	Marker	Chr.	Pos. [bp]	P value	Frequency
Seedling yield	-	-	-	-	-
P concentration seedling	AX-90559399	10	62,300,773	1.18E-11	7
	AX-90549380	6	103,112,536	1.13E-07	1
	AX-91363640	10	18,983,353	2.69E-07	1
	AX-90528468	1	2,106,411	2.82E-06	1
	AX-90539192	3	155,496,851	3.46E-06	1
Stover yield	AX-90551406	7	85,298,782	4.08E-07	1
P concentration stover	AX-90561279	4	238,100,142	3.13E-10	4
	AX-90571724	3	29,590,115	7.13E-10	2
	AX-90540452	3	232,646,913	2.68E-06	1
Grain yield	AX-90538422	3	112,492,593	1.93E-06	2
P concentration grain	AX-90546602	5	140,470,238	3.31E-07	3
P content grain	AX-90554163	2	119,423,856	7.32E-09	1
	AX-90538422	3	112,492,593	1.20E-08	2
	AX-90547819	5	215,515,596	2.78E-07	1
	AX-90565978	6	98,782,602	6.84E-07	1
PUE seedling	AX-90559399	10	62,300,773	4.63E-19	7
	AX-90552092	7	122,960,118	4.01E-08	1
PUE grain	AX-90559399	10	62,300,773	2.37E-07	7
	AX-90546602	5	140,470,238	4.76E-07	3
PUE stover	AX-90561279	4	238,100,142	5.60E-08	4
P content seedling	AX-90542610	4	135,840,932	9.51E-08	1
	AX-90559399	10	62,300,773	5.45E-07	7
	AX-90539179	3	155,180,061	1.30E-06	1

P content stover	AX-90527406	2	205,704,579	5.14E-07	1
	AX-91348995	4	224,627,185	1.06E-06	1
	AX-90559399	10	62,300,773	1.75E-06	7
	AX-91360121	8	135,656,836	2.19E-06	1
P content biomass	AX-90559588	10	71,953,924	1.67E-06	1
PUtE stover	AX-90571337	7	177,638,927	1.40E-13	2
	AX-91354059	6	91,229,070	4.32E-08	1
	AX-90559399	10	62,300,773	2.20E-07	7
	AX-91377974	10	70,052,981	1.05E-06	1
	AX-90558912	10	30,081,724	1.61E-06	1
PUtE grain		-	-	-	-
PutE biomass	AX-90571337	7	177,638,927	2.76E-15	2
	AX-90559399	10	62,300,773	4.71E-15	7
	AX-90571724	3	29,590,115	8.16E-14	2
	AX-90561279	4	238,100,142	1.21E-12	4
	AX-90571133	1	66,654,956	6.66E-09	1
	AX-90542916	4	155,454,018	3.79E-07	1
	AX-90573683	1	51,465,666	5.60E-07	1
	AX-90550118	6	150,515,669	1.37E-06	1
P ratio	AX-90561279	4	238,100,142	2.59E-08	4
QUTIL	AX-90559602	10	73,628,694	1.29E-08	1
	AX-91332263	3	228,880,245	1.21E-06	1
	AX-90546602	5	140,470,238	1.28E-06	3
PUtE int	AX-91364519	10	73,645,344	7.73E-08	1
P balance	AX-90559588	10	71,953,924	1.67E-06	-

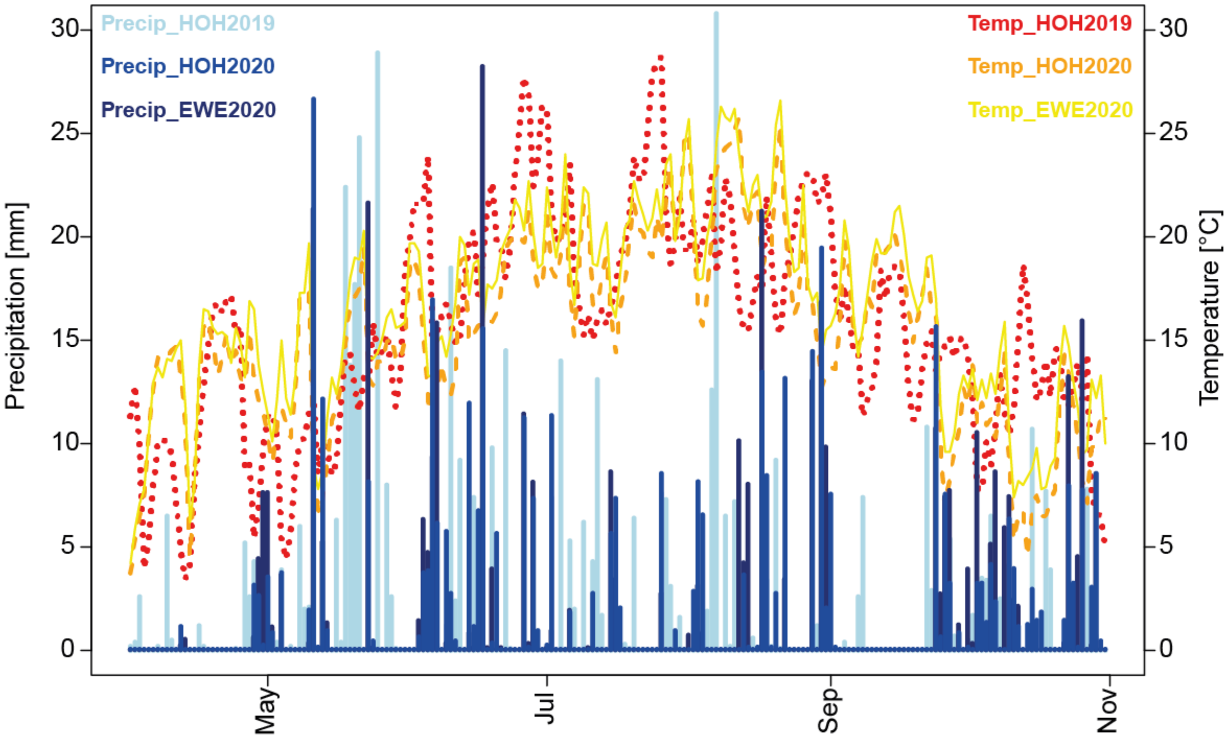


Figure S1 Weather data of the three location-year combinations during the maize growing season. Precipitation is depicted as bar plots, the temperatures as lines.

Discussion

This work was conducted to assess the genetic variation for phosphate efficiency traits in maize and derive implications for practical breeding. In the sister research subject on the Chinese side, our colleagues investigated temperate and tropical/subtropical maize for their adaptation to phosphate tolerance, by focusing on low P soil conditions (Li et al. 2021a; Li et al. 2021b; Li et al. 2019a). The aim of this research was to focus on DHs from Flint landraces, Flint and Dent elite lines, as well as mid-early to mid-late hybrid cultivars currently on the market and assess them for phosphate efficiency under well-supplied soils commonly found in Central Europe.

The key findings of our work at hand are:

(1) Genotypes show different response to the application of starter fertilizers in early developmental stages
(2) The environment has a significant effect on the impact of starter fertilizers
(3) Phenomic selection is a useful and cost-efficient tool to predict traits like phosphorus concentration and grain yield
(4) Population structure does not bias the ability of phenomic selection to predict target traits
(5) DHs from Flint landraces offer valuable allelic diversity with regard to phosphate efficiency during the seedling stage
(6) Genomic selection can complement phenotypic selection for phosphate efficiency traits in a meaningful way
(7) Phosphate efficiency, including its definition and interpretation, is largely context dependent

The following paragraphs should elucidate the meaning of this research: what are the factors that need to be considered when breeding for phosphate efficiency on highly supplied soils?

Interaction of P with other elements

P cannot and should not be regarded separately due to the various interactions that are known to exist with other plant nutrients. We observed in our commercial hybrid trial the biggest effect of starter fertilizers for the diammonium phosphate treatment, in particular for early plant height measurements (Weiß et al. 2021). This synergistic effect of nitrogen and phosphorus has been reported in several studies and it is a well-established fact that the combination of N + P is more effective than their single application (Bello and Yusuf 2021; Nkebiwe et al. 2016). The reason for this lies in the acidification of soil through ammonium and the thereby caused increased P availability for root uptake (Vogel et al. 2020; Jing et al. 2012). It is even said that eventually it is the availability of nitrogen that determines phosphate efficiency as *the* crucial limiting factor for grain yield in most cases (Kenea et al. 2021; Mpanga et al. 2019; Mascagni Jr. and Boquet 1996).

Nevertheless, since we investigated the treatments P in the form of TSP and N in the form of CAN fertilizers also separately, we observed only significant effects on grain yield if both nutrients were combined, illustrating that it is indeed the combination of nitrogen and phosphorus, which leads to a reinforced positive effect.

In contrast to nitrogen in form of ammonium, zinc and P are antagonistic players (Wang et al. 2017; Zhang et al. 2017). Both can form insoluble complexes in soil and if one of the nutrients is applied as fertilizers, less root exudates are released and therefore the respective other nutrient is worse available and to a lesser extent taken up by plants. This is why high P application rates have been shown to impede zinc uptake and vice versa (Saboor et al. 2021; Zhang et al. 2021b). Moreover, P in the grain in the form of phytate hinders the uptake of iron and zinc in humans and animals, which poses especially in developing countries a severe problem (Akhtar et al. 2018).

We also analyzed 16 chemical elements in hybrid maize grain and observed strong positive correlations of P concentration with the micronutrients magnesium, potassium, and manganese (Weiß et al. 2021, Figure S4). This is in line with other studies (Lim and Yi 2019). Magnesium is generally located in deeper soil levels than P and the magnesium-phosphate ratio is associated with root traits resulting in a synergism between the two elements (Weih et al. 2021). Despite the positive correlation of P and potassium in the grain, various foliar P application levels during the four-leaf stage led to higher P but lower potassium concentrations in shoot and root tissue (Görlach and Mühling 2021). Interestingly, manganese concentration in the leaves has been shown to serve as a signature for P-mobilizing carboxylates in the rhizosphere (Lambers et al. 2021). These described relationships underline that P interacts closely with other plant nutrients and the interactions change dynamically over time and with tissue type.

Lately, the fertilization with the micronutrient silicon has gained increasing attention in practical farming (Breuer 2021). Co-fertilization with silicon is deemed suitable to increase P availability since both elements have a very similar molecular size and silicon can thus replace the P locked in the form of complexes in the soil (Frank Stephano et al. 2021; Kostic et al. 2017). This results in a positive effect of silicon fertilization on chilling tolerance during seed germination and seedling growth of maize (Sun et al. 2021; Moradtalab et al. 2018).

Last but not least, the combination of mineral and organic nutrient sources results in higher yields and is deemed beneficial for soil fertility (Bello and Yusuf 2021; Xin et al. 2017). The application of organic manure generally implies higher available P contents (Abid et al. 2020).

Taken together, an integrated nutrient management, considering co-fertilization with other nutrients as well as combinations with organic fertilizers, should be aimed for in order to ensure a sustainable P fertilization.

When do starter fertilizers pay off?

It is common knowledge among farmers and researchers alike that starter fertilizers are especially worthwhile to enhance early development under cold and wet conditions (Pedersen et al. 2019; Bundy and Andraski 1999). For our trials, this was the case for the field season 2019, in which temperature at the location Hohenheim deviated by – 2.6°C and precipitation by + 89.5 % from the long-term mean in May, when maize usually emerges in Germany. Such conditions can lead to micronutrient limitations in maize as low root zone temperatures result in a reduced uptake of minerals by roots due to a reduced activity of enzymes and transporters (Moradtalab et al. 2018; Zhou et al. 2021). The three-leaf to six-leaf stage of maize is reported to be crucial since grain P storage is depleted then and the maize seedling has to switch to heterotrophic nutrition (Bello 2021; Lu et al. 2020; Messiga et al. 2020). For this reason, we regularly measured plant height as indicator for early development during this phase (Figure 3, activity [4]). Biggest differences between treatments with and without TSP starter fertilizer were observed around 60 days after sowing (DAS) for lines and hybrids alike (Figure 8). Besides the evident fact that lines are significantly smaller than hybrids, lines showed an overall higher phenotypic variation for the trait plant height and moreover, lines seem to respond on average stronger (+ 6 cm) to the starter fertilizer application compared to hybrids (+ 2 cm).

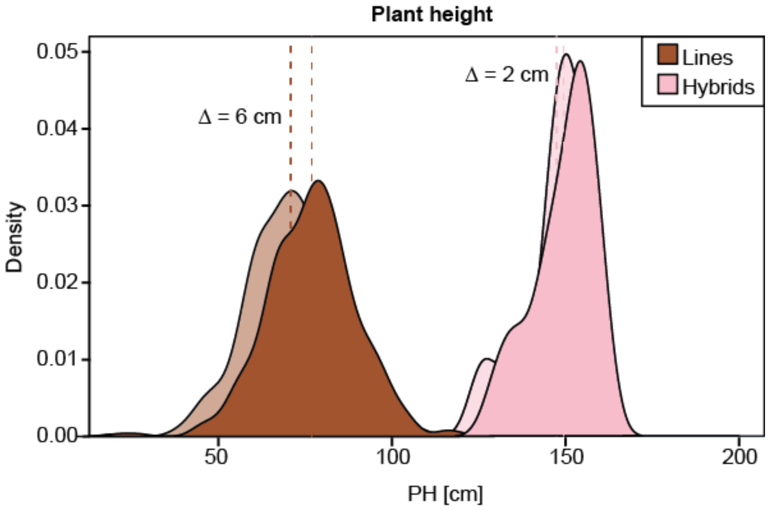


Figure 8 Plant height differences between control (transparent color) and TSP starter fertilizer (opaque color) measured 63 days after sowing (DAS) in the lines trial and 59 DAS in the hybrid trial for Hohenheim, 2019.

In contrast to this, maturity plays a more important role in hybrids compared to lines (W. Schmidt 2021, personal communication, 10 November). Grain maize breeders are usually interested in the relationship between grain yield (GY) and grain dry matter content (GDM) to ensure no gradual shift towards later maturing genotypes but considering maturity-corrected yields as selection criterion. Hence, genotypes above the regression line are usually selected (Figure 9). Maturity, expressed as GDM at harvest (Figure 9a) or alternatively as flowering date, e.g. days to silking (Figure 9b), is tightly related to final grain yield. Later maturing hybrids, indicated by lower grain dry matter contents at harvest or more days to silking, generally result in higher GY as they have more time for light interception (Gasura et al. 2013). Although for our GDM data the trends of no starter fertilization (Co) and TSP are similar (Figure 9a, slope Co = -0.06, slope TSP = -0.07), the TSP regression line is shifted to the right (Figure 9a, intercept Co = 15.63, intercept TSP = 16.43). This also shows that later genotypes have the tendency to benefit slightly more. The effect of TSP starter fertilizer on grain yield was non-significant with an increase of 0.14 t dry matter/ha (Weiß et al. 2021). Nevertheless, the here shown regression line for GY and GDM reveals that for the same average GY in the control treatment of 11.75 t/ha, a TSP starter fertilization results in an increase of 2.71 % GDM compared to the control.

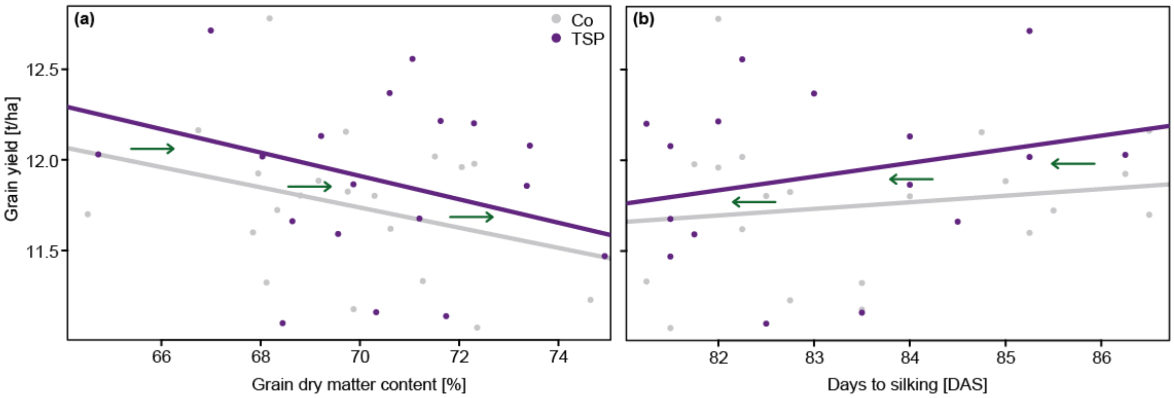


Figure 9 Regression lines between (a) grain yield and grain dry matter content, (b) grain yield and days to silking for best linear unbiased estimates obtained by the multi-environmental hybrid trial in 2019. The control with no starter fertilizer (Co, grey) and the triple superphosphate (TSP, purple) treatment are depicted. Green arrows indicate the shift from Co to TSP starter fertilizer application.

This circumstance is somewhat hidden in the data as the comparison of means between control (mean = 69.70 %) and TSP treatment (mean = 70.39 %) for GDM did not result in a significant difference. However, the decisive quantity is the combination of grain yield and grain dry matter. Eventually, farmers growing maize for grain usage pay drying costs per unit fresh weight. Assuming a GDM of 78 %, which is normally envisaged for maize grain harvest in Germany, grain has to be dried down to 14 %, which corresponds to an 8 % increase in GDM. Therefore, the resulting decrease in drying costs of around 3 % is not to be neglected, especially having current energy prices in mind. Likewise, we observed a shift towards earlier flowering when maize was fertilized with TSP by approximately one day. However, compared to studies with severe, hence real P deficiency and thereof resulting flowering delays in sorghum (*Sorghum bicolor* L. Moench) of up to 10 days (Leiser et al. 2012), the here observed shift is rather minor. For some target environments, the selection for an accelerated early development and therefore earlier flowering may not be desirable (Hölker et al. 2019a). Generally speaking though, an advancement of flowering time through the application of starter fertilizers could imply an escape of water deficit periods or other stresses (Mascagni Jr. and Boquet 1996). We can confirm based on our hybrid trial data that phosphate efficiency is confounded with developmental and maturity genes and thereby adding to its complexity (Bovill et al. 2013). As we have seen, a higher GDM at harvest basically means more grain dry matter grain yield and advancing the flowering time can be a decisive factor towards the end of a field seasons with challenging conditions. These implications may be the tipping point for making maize cultivation possible in locations, which were formerly deemed unsuitable to grow maize due to economic or ecological reasons (F.J. Mauch 2022, personal communication, 7 April).

Collectively, the application of starter fertilizers is relevant especially at early stages under low soil temperatures. Moreover, it may be not the pure yield advantage that counts for the farmer but the potential reduction of drying costs. Whether the current high fertilizer prices can be compensated by the abovementioned advantages has to be thoroughly evaluated by business economists or, more likely, will be decided by farmers themselves.

Meaning of the selection environment for phosphate efficiency breeding

Ultimately, breeders have to ask themselves what the target environment is they are breeding for. In the case of this work, the target environment was characterized by a high soil P status (Weiß et al. 2021). Most studies investigating phosphate efficiency perform their experiments under different levels of P supply in order to compare low versus high conditions and then derive the so called P tolerance for different genotypes (Li et al. 2021a; Li et al. 2021b; Rose et al. 2016; Leiser et al. 2015; Leiser et al. 2014a; Wissuwa and Ae 2001). Having said this, it has to be stated that P-deprived soils, which would be necessary to realize such different P treatments, are not easily to be found in Central Europe. From a breeding perspective though, different levels of P supply only need to be analyzed and reported with regard to a potential genotype-by-P-level interaction (Leiser 2014, p.8). In environments which are highly P deficient, the application of P fertilizers leads to a significant, but generally low genotype-by-P-level interaction (Leiser et al. 2014a; Leiser et al. 2012). Rose et al. (2016) did not observe any significant genotype-by-P-level interaction, neither in hydroponic nor on soil-based experiments. In our hybrid trials taking place in sufficiently to highly P supplied soils, no significant interaction between genotype and starter fertilization was observed (Weiß et al. 2021). Most likely, we already surpassed the crossover point of different P conditions (Leiser 2014) and therefore the slope between high and very high P supply is minor, resulting in a non-significant genotype-by-P-level interaction. As a consequence, subsequent studies investigating phosphate efficiency under well-supplied conditions only considered one P level, namely the control with no extra P input in from of starter fertilizers (Weiß et al. 2022; Weiß et al. unpublished). This approach is deemed adequate in light of the broad-sense heritability calculation going back to Hallauer (2010):

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{g\times y}^2}{n_y} + \frac{\sigma_{g\times l}^2}{n_l} + \frac{\sigma_{g\times y\times l}^2}{n_{y} * n_l} + \frac{\sigma_\varepsilon^2}{n_{y} * n_{l} * n_r}} \qquad (1)$$

With σ_g^2, $\sigma_{g\times y}^2$, $\sigma_{g\times l}^2$, $\sigma_{g\times y\times l}^2$, σ_ε^2 denoting the genotypic, genotype-by-year, genotype-by-location, genotype-by-year-by-location, and error variance terms and n_y, n_l and n_r denoting the number of years, locations and replications, respectively. Taking into account that genotype-P-level interactions do not seem to be relevant in the given framework, the different P treatments can be seen as kind of replicate, entering the formula only in one term. Therefore, it is more meaningful to increase the number of environments by adjusting the allocation of resources towards more locations and years instead of different P-treatments for future phosphate efficiency research taking place in well-supplied soils.

Another factor, which has to be considered when choosing the selection environment for phosphate efficiency breeding, is the genotype-by-environment interaction. We have seen, also in former parts of this thesis, that the soil in all its complexity is a major driver of P performance (Hinsinger 2001). Moreover, especially low temperatures are known to have an impact on early development of maize, a factor that is obviously highly depending on location and year. Flint genotypes are attributed to react more to environmental effects than Dents do (W. Schipprack 2019, personal communication, 15 July). The genotype-by-location interaction variance was highly significant for most traits under investigation (Weiß et al. 2021). Additionally, the lowest ratio between genotypic and genotype-by-environment variance was observed for the trait P content of the seedling, which underlines the sensitivity of seedlings to environmental influences (Weiß et al. unpublished). All other ratios for phosphate efficiency indicators showed levels above 1, which emphasizes the meaning of the pure genotypic component nonetheless.

Lastly, research has been conducted to perform selection under artificial conditions to realize a higher selection gain (Jähne et al. 2020). However, it should be considered that maize plants are rather tall and different story layers optimizing space issues are probably not feasible. This is one of the reasons why in maize pot experiments often only youth developmental analyses are performed (e.g. Ma et al. 2021; Azevedo et al. 2015). Liu et al. (2017) pointed out that it is problematic to extrapolate results obtained under one growing system to another, which should be also kept in mind.

Moreover, there has been a lot of discussion going on regarding the meaning of environments in the phenomic selection (PS) approach, also applied in this work to predict phosphate efficiency-relevant traits (Weiß et al. 2022). Environments have a crucial impact on PS predictive ability and it was recommended to use environments exposed to stresses as they will increase phenotypic differentiation and thereby result in better predictive abilities (Lane et al. 2020). Zhu et al. (2021) reported no satisfying results if the training and prediction set for performing PS are grown in different environments. We can infer that phenomic predictions generally work better if the material is grown in the same environment or if several environments are combined together (Robert et al. 2022).

Overall, enviromics should be more considered and become a routine part in breeding pipelines (Cooper and Messina 2021). The choice of type and number of environments has to be undertaken with great care depending on the targeted environment. More precisely, the contained interactions between genotypes and environments have to be considered in order to maximize heritability and thus selection gain.

The implications of working with diverse breeding material

In the underlying work, we investigated phosphate efficiency-related measures in 20 commercial hybrids, 100 elite lines each of the Flint and Dent heterotic pool, as well as 200 DH lines from landraces. The most pronounced differences between the material groups can be observed for the trait grain yield (Figure 10).

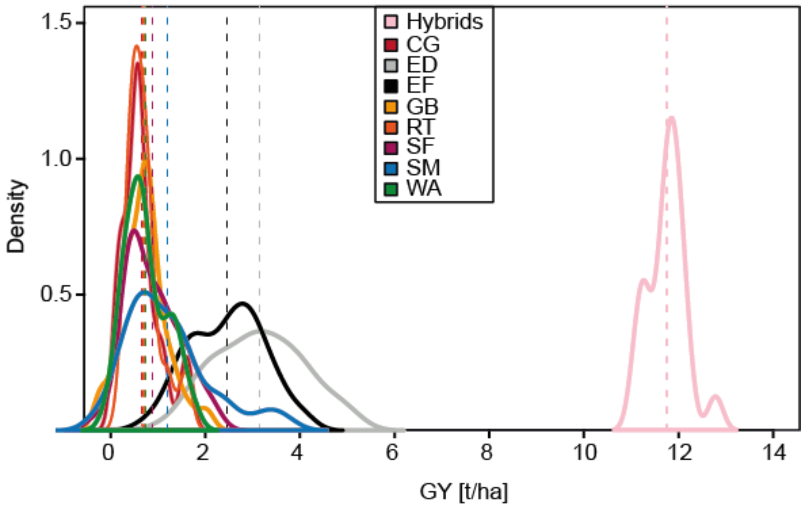


Figure 10 Grain yield (GY [t/ha]) best linear unbiased estimates obtained by multi-environmental trials for the different material groups investigated in this work. Groups are abbreviated as Campan-Galade (CG), elite Dents (ED), elite Flints (EF), Gelber Badischer (GB), St. Galler Rheintaler (RT), Strenzfelder (SF), Satu Mare (SM), and Walliser (WA). Values are based on control plots with no extra starter fertilization.

As expected, DH lines from landraces are inferior to the breeding pools elite Dents and elite Flints and in a completely different range than commercial hybrids. Previous studies reported a smaller than expected yield gap between elite lines and some landraces, but clear superiority of recent elite material (Hölker et al. 2019b). In line with our results, Böhm et al. (2017) described that several genotypes of the landrace Satu Mare (SM) produced similar yields compared with the best elite lines. Next to the undoubtedly important trait grain yield, we also investigated numerous other parameters, for which landraces showed a huge variation and also significant differences among populations (Weiß et al. 2022; Weiß et al. unpublished). Especially seedling traits have shown promising characteristics with regard to phosphate efficiency (Weiß et al. unpublished). Nonetheless, we recommend to use yield and P concentrations of different tissues as straight forward parameters to select for phosphate efficiency. Therefore, selection in diverse material should always account for performance discrepancies among different groups. Moreover, as discussed in a previous section about maturity, when selecting for yield traits at harvest, we have to pay attention to not gradually and unconsciously select later maturing and therefore better performing genotypes.

The suitability to use DH lines from landraces for GWAS and the resulting high mapping resolution has been pointed out in several studies (Würschum et al. 2022; Mayer et al. 2020). Therefore, landraces hold great potential for detecting underlying causal genes of relevant traits in maize breeding. Although ideal prerequisites were thus given for association mapping, we could not identify consistent marker-trait associations across the phosphate efficiency traits under investigation (Weiß et al. unpublished). Clearly, such new allelic diversity detected in landraces would in advance need to be introduced by marker-assisted backcrossing or new breeding technologies (Ran et al. 2013) in order to avoid yield reductions.

Last but not least, one main finding of our work was the suitability of genomic selection (GS) for the prediction of phosphate efficiency measures. We observed that GS works best for the group of elite Dents (Weiß et al. unpublished). Besides the generally higher genetic diversity in the Dent compared to the Flint pool (Rebourg et al. 2003), which has been addressed in the introduction chapter, single nucleotide polymorphism (SNP) marker arrays were originally developed to detect polymorphisms in Northern American Dents (Frascaroli et al. 2013). Although the resulting bias cannot be seen in the absolute number of polymorphic markers, the number of markers being excluded after imputation due to a minor allele frequency (MAF) of below 5 % was substantially higher for Flint elite lines (12.7 %) compared to Dents (8.5 %). Elite Flint markers generally occured with lower minor allele frequencies (MAF < 0.2 = 57 %) than elite Dents (MAF < 0.2 = 41 %). This shift of the elite Flint markers towards lower minor allele frequencies may be one potential explanation for higher prediction accuracies obtained in elite Dents. However, the in our work applied ridge-regression best linear unbiased prediction method should not be affected by the quantity of genetic markers with low minor allele frequency as much as other GS methods (Zhang et al. 2019).

In conclusion, landraces show promising characteristics with regard to phosphate efficiency-related traits and broadening the genetic basis of the elite material. At the same time, yield gaps and differences among subgroups have to be taken into consideration when selecting for phosphate efficiency.

Prediction and interrelations of P concentration

Previously, it was elaborated why P concentration plays an important role in breeding for an improved phosphate efficiency. Several studies have demonstrated the negative relationship between P concentration and yield as a consequence of a dilution effect (Arrobas et al. 2022; Hertzberger et al. 2021). To account for this effect, the product of yield and P concentration is usually evaluated, resulting in the parameter P content. In our dataset, the general trend of a decreasing P concentration with increasing yield could be well observed for hybrids and lines alike (Figure 11).

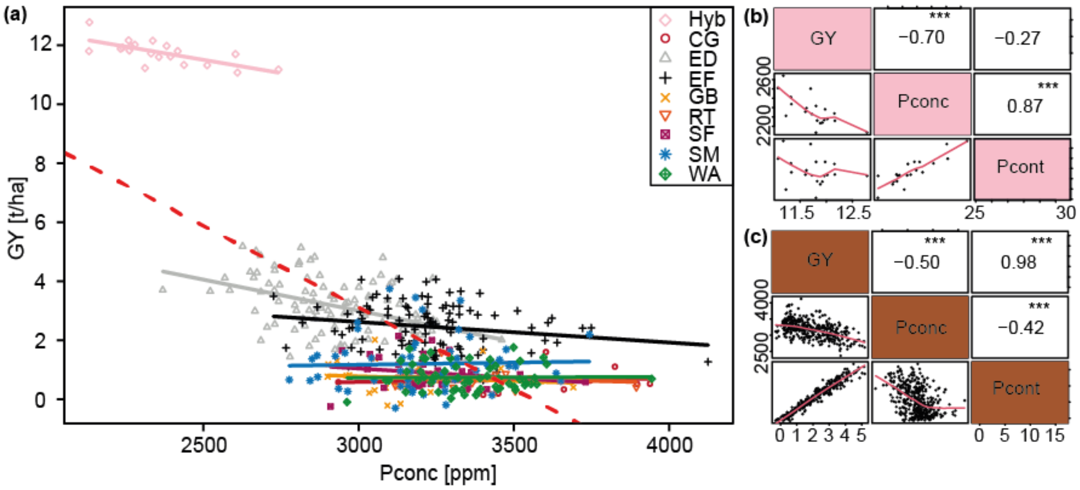


Figure 11 Relationship between best linear unbiased estimates for grain yield (GY [t/ha]) and P concentration in the grain (Pconc [ppm]), (a) split up into hybrids and single DH lines from landraces with the overall trend between both traits indicated as dashed red line. Groups are abbreviated as commercial hybrids (Hyb), Campan-Galade (CG), elite Dents (ED), elite Flints (EF), Gelber Badischer (GB), St. Galler Rheintaler (RT), Strenzfelder (SF), Satu Mare (SM), Walliser (WA). P content (Pcont [kg/ha]) was added for the correlation matrices of (b) hybrids and (c) all lines jointly. Values are obtained from multilocational field trials under control conditions without extra starter fertilization.

Notably, the extent of this negative relation seems to vary largely depending on the material group (Figure 11a): the higher the yield level, the stronger the dilution effect for P concentration. When looking at hybrids and lines separately, we make the interesting observation that P content is in the case of hybrids mainly driven by the variable P concentration with a correlation coefficient of r = 0.87*** (Figure 11b), whereas grain yield basically determines P content for the line genotypes with r = 0.98***. This may be due to the fact that P concentration in the case of hybrids and grain yield in the case of lines contribute as bigger source of variation to P content, respectively.

P concentration in our maize samples was analyzed by means of X-ray fluorescence (XRF). The sample throughput for measuring P concentration by XRF amounts to 90 samples per day (C. Maus and R. Bauer 2021, personal communication, 4 February). With an average sample volume of more than 5 000 units per field season, this means around three months of solely analyzing samples for P concentration - without taking sample preparation into account. Therefore, a reliable *prediction* of P concentration is desirable. Genomic selection is successfully applied in maize breeding programs and yields accuracies which correspond to phenotyping unreplicated field trials in three to four locations (Zhao et al. 2012). The rather novel approach of phenomic prediction has proven useful in the prediction of complex traits (Robert et al. 2022; Zhu et al. 2021; Rincent et al. 2018). We applied this method using near-infrared spectroscopy (NIRS) data instead of marker data and obtained high prediction accuracies for P concentration (Weiß et al. 2022). For the case of P concentration, we highlighted the advantage of PS over GS with regard to robustness towards population structure. NIRS data acquisition is twice or trice as fast as XRF measurements and in particular the costs between the two machines differ significantly. Evidently, certain samples have to be wet chemically analyzed for P concentration, also in the case of phenomic prediction, in order to set up a calibration. Based on the abovementioned assumptions, however, PS holds great potential to save costs and time and enables the screening of more candidates during the breeding process.

We were further interested to compare which 10 % best performing genotypes are identified in PS versus GS based on the predicted grain yield (results not shown). As a matter of fact, different genotypes would be selected if marker or NIRS data are used as predictors. The reason for this may be that phenomic selection captures environmental in addition to genotypic effects (Rincent et al. 2018). Of course, breeders are interested primarily in the genetic component of a trait. Nevertheless, PS is deemed suitable for predicting large numbers of genotypes at earlier stages of the breeding pipeline and by this discarding the worse half for example.

Moreover, the prediction of phytate being the main constituent of total P in the maize grain, was tested. Phytate measurements by wet chemical analysis are very laborious. Moreover, it should be noted that IP6 is sensitive to the temperature under which maize kernels are dried. Therefore, only data from the line field trial in 2019 were available, when maize samples were additionally dried at 40°C. If we use conventional NIRS calibration we obtained an R^2 of 0.76. The mean predictive ability based on the 100 wet-chemically analyzed maize samples yielded 0.61 for phenomic and 0.52 for genomic prediction. Including samples that were dried at 110°C resulted in considerable decreases of the predictive ability though. Thus, selection for phytate concentrations and the IP6 to total P ratio are best performed at later stages of the breeding pipeline when only comparatively few candidates are left for more detailed assessments and an additional lower drying temperature can be afforded.

As shown above, P concentration and its correlation with yield and P content is strongly determined by the breeding material. Considering that phosphate efficiency is specifically important in low-P soils, which are predominantly encountered in Africa’s and South America’s highly weathered soils, PS might represent an attractive solution in this context as generally less resources for phosphate efficiency breeding are disposable. Lastly, we have seen that phytate can only to a limited extent be predicted by genomic and phenomic approaches due to its high sensitivity with regards to drying temperature and storage.

How to increase phosphate efficiency in Central Europe

Why phosphate efficiency will most likely play a bigger role in agriculture and hence plant breeding was comprehensively explained in previous sections of this work. What makes the target environment of Central Europe distinct is that phosphate efficiency has not been in the focus of breeding activities so far, because we mostly deal with highly P supplied soils. As a consequence, the goal in this setting must be to minimize extra mineral fertilizer input while maintaining high yield levels.

Based on this statement, the first follow-up action for breeders should be that breeding nurseries take place under low input regimes (Weiß et al. 2021). Thereby, we can identify and select promising candidates under conditions that are to be expected in the near future. To the best of our knowledge, this is already the case for national variety trials. However, enquiries at different breeding companies revealed that starter fertilization is oftentimes applied as common practice standard procedure. This has to change if we want to develop varieties that perform well under reduced P fertilizer conditions.

Next, we showed the potential of phenomic selection to predict complex traits relevant for assessing phosphate efficiency, such as P concentration or grain yield (Weiß et al. 2022). One huge advantage lies in the fact that population structure is not having an impact on prediction outcomes when applying NIRS data as predictors. In contrast to this, we need to correct for population structure when using marker data as predictors even when working in an elite maize breeding population (Liu et al. 2011). Hence, phenomic selection is well suited to produce robust results in diverse material, which may in maize be for instance the case during pre-breeding activities. As breeding always needs to be cost-efficient, it is of great importance to allocate resources in the best possible way. Here, we proposed to take advantage of the comparatively cheap and time-efficient method of PS to predict P concentration and grain yield for a large number of individuals as this is the case early on in breeding programs and negatively select for the worst fraction. At the end of the day, the application of PS is not an either-or-decision. We want to rather point out how PS and GS are both useful tools for the breeders and can be applied in a complementary way.

And third, this work demonstrated how phosphate efficiency is a complex trait to breed for (Weiß et al. unpublished). Breeders prefer to work with traits that show a small environmental dependency of genotypes and that are ideally based on a few causal genes. In the case of phosphate efficiency-related traits, however, we reported a strong environmental effect and the genetic nature seems to be quantitative in the setting of high and very high P levels of the soil. Therefore, we advise to use the direct and primary traits P concentration and yield in the corresponding tissues to capture phosphate efficiency and rely on genomic selection rather than marker-assisted selection to improve these generally quantitatively inherited traits.

Taken together, breeding companies have to come up with solutions to make their varieties less dependent on fertilizer inputs. Including phosphate efficiency traits in the selection criteria is feasible and we observed sufficient genetic variation to develop phosphate-efficient varieties under Central European conditions.

Limitations and recommendations

We have seen in the course of this study that the environment, namely location-specific soil characteristics and year-specific weather conditions, has a decisive influence on the observed phosphate efficiency of maize. Due to this fact, multi-year field trials over a longer period including more locations rather than different P treatments would be of interest to quantify the environmental effect on the genotypes and derive robust conclusions about their performance. It has been emphasized that long term assessments which take the environmental variability of genotypic responses into consideration would be highly desirable (Bovill et al. 2013).

Furthermore, it is evident that roots as interface between plants and soil play a crucial role in phosphate efficiency performance. Especially in the light of climate change, future research should focus more on underground traits as an improvement of these is expected to be associated with general abiotic stress tolerance (Lynch 2007).

One major outcome of this work was the demonstration of successfully applying phenomic selection based on wavelength data obtained by a NIR spectrometer to predict phosphate efficiency-relevant traits in maize. We have been also collaborating with the agricultural engineering group of our university, who monitored our field trials with unmanned aerial vehicles. A next step could therefore be to collect near-infrared data by means of drones, predict traits via phenomic selection based on this, and save even more valuable time and resources during the breeding process.

Last, but not least, we made clear in various places that the assessment of phosphate efficiency traits under well-supplied P levels comes along with certain consequences. One is that we cannot detect great phenotypic differences between different treatments on an already high P level to start with. Additionally, we were not able to detect consistent marker-trait associations across phosphate efficiency traits. However, we expect nutrient efficiency in crops to gain more attention in the future due to the development of fertilizer prices described at the beginning. For this reason, we identified reasonable measures, which can help to meet the requirements for the successful breeding of phosphate-efficient maize under European conditions.

Summary

Why should plant breeders in Central Europe care about phosphate efficiency? Soil phosphorus levels have mostly reached high to very high levels over the last decades in intensively farmed, livestock-rich regions. However, the European Union demands a restructuring of the agricultural production systems through setting ambitious goals envisaged in the Farm to Fork Strategy. By 2030, fertilizer use should be reduced by 20 %, nutrient losses by at least 50 %. As a consequence, farmers have to be even more efficient with crop inputs, among them the globally limited resource of phosphorus fertilizers, while maintaining high yields. Plant breeding means thinking ahead. Therefore, phosphate-efficient varieties should be developed to help farmers meet this challenge and reduce the need for additional fertilizers. One prerequisite to reach this target is that genotypic variation for the relevant traits is available. Moreover, approaches that assist selection by accurate but also time- and resource-efficient prediction of genotypes are highly valuable in breeding. Finally, the choice of the selection environment and suitable trait assessment for the improvement of phosphate efficiency under well-supplied conditions, need to be elaborated.

In this dissertation, a diverse set of maize genotypes from ancient landraces to modern hybrids was investigated for phosphate efficiency-related traits under well-supplied P soil conditions. Multi-environmental field trials were conducted in 2019 and 2020.

The reaction to different starter fertilizer treatments of the 20 commercially most important maize hybrids grown in Germany was studied. In the hybrid trial, the factor environment had a significant effect on the impact of starter fertilizers. Especially in early developmental stages genotypes showed a different response to the application of starter fertilizers. On the overall very well-supplied soils, we observed no significant genotype-by-starter fertilizer interaction. Nonetheless, we identified hybrids, which maintained high yields also if no starter fertilizer was provided. Thus, it seems that sufficient variation is available to select and breed for phosphate efficiency under reduced fertilizer conditions.

Furthermore, the concept of phenomic prediction, based on near-infrared spectra instead of marker data to predict the performance of genotypes, was applied to 400 diverse lines of maize and compared to genomic prediction. For this, we used seed-based near-infrared spectroscopy data to perform phenomic selection in our line material, which comprised doubled haploid lines from landraces and elite lines. We observed that phenomic prediction generally performed comparable to genomic prediction or even better. In particular, the phenomic selection approach holds great potential for predictions among different groups of breeding material as it is less prone to artifacts resulting from population structure. Phenomic selection is therefore deemed a useful and cost-efficient tool to predict complex traits, including phosphorus concentration and grain yield, which together form the basis to determine phosphate efficiency.

Lastly, 20 different indicators for phosphate efficiency were calculated, the genetic variation of the different measures present in this unique set of lines was quantified, and recommendations for breeding were derived. Of the different measures for phosphate efficiency reported in literature, Flint landraces demonstrated valuable allelic diversity with regard to phosphate efficiency during the seedling stage. Due to the highly complex genetic architecture of phosphate efficiency-related traits, a combination of genomic and phenotypic selection appears best suited for their improvement in breeding. Taken together, phosphate efficiency, including its definition and meaning, is largely dependent on the available phosphorus in the target environment as well as the farm type, which specifies the harvested produce and thereby the entire phosphorus removal from the field.

In conclusion, future maize breeding should work in environments that are similar to the future target environments, meaning reduced fertilizer inputs and eventually lower soil P levels. Our results demonstrate that breeding of varieties, which perform well without starter fertilizers is feasible and meaningful under the well-supplied conditions prevalent in Central Europe. For the improvement of the highly complex trait phosphate efficiency through breeding we recommend to apply genomic and phenomic prediction along with classical phenotypic screening of genotypes and by this making our food systems more resilient towards upcoming challenges in agriculture.

Zusammenfassung

Warum sollten sich Pflanzenzüchter:innen in Mitteleuropa mit Phosphateffizienz beschäftigen? Der Phosphorgehalt im Boden ist in den letzten Jahrzehnten vor allem in intensiv bewirtschafteten viehreichen Regionen auf ein hohes bis sehr hohes Niveau angestiegen. Die Europäische Union formuliert jedoch in der „Farm to Fork“-Strategie ehrgeizige Ziele für eine Umstrukturierung der landwirtschaftlichen Produktionssysteme. Bis 2030 soll der Düngemitteleinsatz um 20 % und die Nährstoffverluste um mindestens 50 % reduziert werden. Das bedeutet, dass die Landwirte noch effizienter mit dem Einsatz von Betriebsmitteln umgehen müssen, insbesondere mit der weltweit begrenzten Ressource von Phosphordüngern, bei gleichzeitig weiterhin hohen Erträgen. Pflanzenzüchtung bedeutet vorausschauend zu denken. Daher sollten phosphateffiziente Sorten entwickelt werden, die den Landwirten helfen, diese Herausforderung zu meistern und den Bedarf an zusätzlichen Düngemitteln zu verringern. Eine Voraussetzung, um dieses Ziel zu erreichen, ist, dass genotypische Variation für die relevanten Merkmale vorhanden ist. Darüber hinaus sind Ansätze, die die Selektion durch eine genaue, aber auch zeit- und ressourceneffiziente Vorhersage von Genotypen unterstützen, in der Züchtung sehr wertvoll. Außerdem müssen die Wahl der Selektionsumwelt und eine geeignete Merkmalserfassung für die Verbesserung der Phosphateffizienz unter gut versorgten Bedingungen näher beleuchtet werden.

In dieser Dissertation wurde eine Reihe von Maisgenotypen, von alten Landrassen bis hin zu modernen Hybriden, bezüglich Phosphateffizienz-Merkmalen auf gut mit P versorgten Böden untersucht. In den Jahren 2019 und 2020 wurden mehrortige Feldversuche durchgeführt.

Untersucht wurde die Reaktion der 20 kommerziell wichtigsten in Deutschland angebauten Maishybriden auf unterschiedliche Unterfußdüngungen. In dem Hybridversuch hatte der Faktor Umwelt einen signifikanten Einfluss auf die Wirkung von Unterfußdüngern. Insbesondere in frühen Entwicklungsstadien reagierten die Genotypen unterschiedlich auf die Gabe von Unterfußdüngern. Auf den insgesamt sehr gut versorgten Böden beobachteten wir keine signifikante Interaktion zwischen Genotyp und Unterfußdünger. Dennoch konnten wir Hybriden identifizieren, die auch ohne Unterfußdünger hohe Erträge erzielten. Es scheint also genügend Variation vorhanden zu sein, um unter reduzierten Düngebedingungen auf Phosphateffizienz zu selektieren und zu züchten.

Darüber hinaus wurde das Konzept der phänomischen Vorhersage, welches auf Nahinfrarotspektren anstelle von Markerdaten zur Vorhersage der Leistung von Genotypen basiert, auf 400 verschiedenen Maislinien angewandt und mit der genomischen Vorhersage verglichen. Hierbei nutzten wir samenbasierter Daten der Nahinfrarotspektroskopie, um phänomische Selektion in unserem Linienmaterial durchzuführen, welches doppelhaploide Linien von Landrassen und Elitelinien enthielt. Wir konnten feststellen, dass die phänomische Vorhersage im Allgemeinen mit der genomischen Vorhersage gleichauf oder sogar besser war. Der phänomische

Selektionsansatz hat insbesondere für Vorhersagen zwischen verschiedenen Gruppen von Zuchtmaterial großes Potenzial, da er weniger anfällig für Artefakte ist, die aus der Populationsstruktur resultieren. Die phänomische Selektion hat sich daher als nützliches und kosteneffizientes Instrument zur Vorhersage komplexer Merkmale erwiesen, einschließlich der Phosphorkonzentration und des Kornertrags, welche zusammen die Grundlage für die Bestimmung der Phosphateffizienz bilden.

Zuletzt wurden 20 verschiedene Indikatoren für Phosphateffizienz berechnet, die genetische Variation der verschiedenen Messgrößen in dieser spezifischen Zusammensetzung von Linien quantifiziert und Empfehlungen für die Züchtung abgeleitet. Von den verschiedenen in der Literatur beschriebenen Maßen für die Phosphateffizienz zeigten Flint-Landrassen eine wertvolle allelische Diversität in Bezug auf die Phosphateffizienz im Keimlingsstadium. Aufgrund der hochkomplexen genetischen Struktur von Phosphateffizienz-Merkmalen, scheint eine Kombination aus genomischer und phänotypischer Selektion am besten geeignet, um diese züchterisch zu verbessern. Alles in allem hängt die Phosphateffizienz, einschließlich ihrer Definition und Bedeutung, weitgehend vom verfügbaren Phosphor in der angestrebten Umwelt sowie vom Betriebstyp ab, da dieser das Erntegut und damit die Gesamtphosphorabfuhr vom Feld bestimmt.

Zusammenfassend lässt sich sagen, dass die zukünftige Maiszüchtung in Umgebungen arbeiten sollte, die den zukünftigen Zielumwelten ähnlich sind, was einen geringeren Düngemitteleinsatz und schließlich einen niedrigeren P-Gehalt im Boden bedeutet. Unsere Ergebnisse zeigen, dass die Züchtung von Sorten, die ohne Unterfußdünger auskommen, unter den in Mitteleuropa vorherrschenden gut versorgten Bedingungen realisierbar und sinnvoll ist. Zur Verbesserung des hochkomplexen Merkmals Phosphateffizienz durch Züchtung empfehlen wir, neben der klassischen phänotypischen Begutachtung von Genotypen auch genomische und phänomische Vorhersagen anzuwenden und damit unsere Nahrungsmittelsysteme widerstandsfähiger gegenüber den kommenden Herausforderungen in der Landwirtschaft zu machen.

References

Abid, Muhammad; Batool, Tahira; Siddique, Ghulam; Ali, Shafaqat; Binyamin, Rana; Shahid, Munazzam Jawad et al. (2020): Integrated Nutrient Management Enhances Soil Quality and Crop Productivity in Maize-Based Cropping System. In *Sustainability* 12 (23), p. 10214. DOI: 10.3390/su122310214.

Agricultural School Apolda (1899): Es fängt damit an, daß am Ende der Punkt fehlt. Stilblüten aus amtlichen und privaten Schreiben. With assistance of Margrit Waas, Emil Waas. 40.th ed. München: Deutscher Taschenbuch Verlag.

Akhtar, Sajjad; Osthoff, Gernot; Mashingaidze, Kingstone; Labuschagne, Maryke (2018): Iron and Zinc in Maize in the Developing World: Deficiency, Availability, and Breeding. In *Crop Sci.* 58 (6), pp. 2200–2213. DOI: 10.2135/cropsci2018.02.0133.

Appelhans, Stefania C.; Barbagelata, Pedro A.; Melchiori, Ricardo J. M.; Gutierrez Boem, Flavio H.; Caviglia, Octavio P. (2021): Is the Lack of Response of Maize to Fertilization in Soils with Low Bray1-P Related to Labile Organic Phosphorus? In *J Soil Sci Plant Nutr* 21 (1), pp. 612–621. DOI: 10.1007/s42729-020-00387-8.

Arrobas, Margarida; Decker, João V.; Feix, Bruna L.; Godoy, Wilson I.; Casali, Carlos A.; Correia, Carlos M.; Ângelo Rodrigues, Manuel (2022): Biochar and zeolites did not improve phosphorus uptake or crop productivity in a field trial performed in an irrigated intensive farming system. In *Soil Use and Management* 38 (1), pp. 564–575. DOI: 10.1111/sum.12704.

Azevedo, Gabriel C.; Cheavegatti-Gianotto, Adriana; Negri, Bárbara F.; Hufnagel, Bárbara; E Silva, Luciano da Costa; Magalhaes, Jurandir V. et al. (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. In *BMC plant biology* 15, p. 172. DOI: 10.1186/s12870-015-0561-y.

Balaban, Nelly; Suleimanova, Aliya; Valeeva, Lia; Chastukhina, Inna; Rudakova, Natalia; Sharipova, Margarita; Shakirov, Eugene (2016): Microbial Phytases and Phytate: Exploring Opportunities for Sustainable Phosphorus Management in Agriculture. In *Biological Sciences Faculty Research*. Available online at https://mds.marshall.edu/bio_sciences_faculty/142.

Barrière, Y.; Alber, D.; Dolstra, O.; Lapierre, C.; Motto, M.; Ordás Pérez, Amando et al. (2006): Past and prospects of forage maize breeding in Europe. II. History, germplasm evolution and correlative agronomic changes. In *Maydica* 51, pp. 435–449.

Bello, Suleiman K. (2021): An Overview of the Morphological, Genetic and Metabolic Mechanisms Regulating Phosphorus Efficiency Via Root Traits in Soybean. In *J Soil Sci Plant Nutr* 21 (2), pp. 1013–1029. DOI: 10.1007/s42729-021-00418-y.

Bello, Suleiman Kehinde; Yusuf, Ado Adamu (2021): Phosphorus influences the performance of mycorrhiza and organic manure in maize production. In *Journal of Plant Nutrition* 44 (5), pp. 679–691. DOI: 10.1080/01904167.2020.1849295.

Bernardino, Karine C.; Pastina, Maria Marta; Menezes, Cícero B.; Sousa, Sylvia M. de; Maciel, Laiane S.; Carvalho, Geraldo et al. (2019): The genetic architecture of phosphorus efficiency in sorghum involves pleiotropic QTL for root morphology and grain yield under low phosphorus availability in the soil. In *BMC plant biology* 19 (1), p. 87. DOI: 10.1186/s12870-019-1689-y.

Bilyera, Nataliya; Hummel, Christina; Daudin, Gabrielle; Santangeli, Michael; Zhang, Xuechen; Santner, Jakob et al. (2022): Co-localised phosphorus mobilization processes in the rhizosphere of field-grown maize jointly contribute to plant nutrition. In *Soil Biology and Biochemistry* 165, p. 108497. DOI: 10.1016/j.soilbio.2021.108497.

Böhm, Juliane; Schipprack, Wolfgang; Utz, H. Friedrich; Melchinger, Albrecht E. (2017): Tapping the genetic diversity of landraces in allogamous crops with doubled haploid lines: a case study from European flint maize. In *Theor Appl Genet* 130 (5), pp. 861–873. DOI: 10.1007/s00122-017-2856-x.

Bovill, William D.; Huang, Chun Y.; McDonald, Glenn K. (2013): Genetic approaches to enhancing phosphorus-use efficiency (PUE) in crops: challenges and directions. In *Crop Pasture Sci.* 64 (3), p. 179. DOI: 10.1071/CP13135.

Brauner, Pedro C.; Schipprack, Wolfgang; Utz, H. Friedrich; Bauer, Eva; Mayer, Manfred; Schön, Chris-Carolin; Melchinger, Albrecht E. (2019): Testcross performance of doubled haploid lines from European flint maize landraces is promising for broadening the genetic base of elite germplasm. In *Theor Appl Genet* 132 (6), pp. 1897–1908. DOI: 10.1007/s00122-019-03325-0.

Breuer, Joachim (2021): Das vergessene Element gegen Stress [The forgotten element against stress]. In *BWagrar* 15, 2021, pp. 18–19.

Bundy, L. G.; Andraski, T. W. (1999): Site-Specific Factors Affecting Corn Response to Starter Fertilizer 12 (4), pp. 664–670.

Campbell, Neil A.; Reece, Jane B. (Eds.) (2009): Biologie. With assistance of Neil A. Campbell, Jane B. Reece, Lisa A. Urry, Michael L. Cain, Steven A. Wasserman, Peter V. Minorsky, Robert B. Jackson. 8.th ed.: Pearson Studium.

Castagno, Luis N.; Sannazzaro, Analía I.; Gonzalez, María E.; Pieckenstain, Fernando L.; Estrella, María J. (2021): Phosphobacteria as key actors to overcome phosphorus deficiency in plants. In *Ann Appl Biol* 178 (2), pp. 256–267. DOI: 10.1111/aab.12673.

Ciampitti, Ignacio A.; Camberato, Jim J.; Murrell, Scott T.; Vyn, Tony J. (2013): Maize Nutrient Accumulation and Partitioning in Response to Plant Density and Nitrogen Rate: I. Macronutrients. In *Agron.j.* 105 (3), pp. 783–795. DOI: 10.2134/agronj2012.0467.

Ciampitti, Ignacio A.; Vyn, Tony J. (2014): Understanding Global and Historical Nutrient Use Efficiencies for Closing Maize Yield Gaps. In *Agron.j.* 106 (6), pp. 2107–2117. DOI: 10.2134/agronj14.0025.

Cooper, James; Lombardi, Rachel; Boardman, David; Carliell-Marquet, Cynthia (2011): The future distribution and production of global phosphate rock reserves. In *Resources, Conservation and Recycling* 57, pp. 78–86. DOI: 10.1016/j.resconrec.2011.09.009.

Cooper, Mark; Messina, Carlos D. (2021): Can We Harness "Enviromics" to Accelerate Crop Improvement by Integrating Breeding and Agronomy? In *Front. Plant Sci.* 12, p. 735143. DOI: 10.3389/fpls.2021.735143.

Das, Debatosh; Paries, Michael; Hobecker, Karen; Gigl, Michael; Dawid, Corinna; Lam, Hon-Ming et al. (2022): PHOSPHATE STARVATION RESPONSE transcription factors enable arbuscular mycorrhiza symbiosis. In *Nat Commun* 13 (1), p. 477. DOI: 10.1038/s41467-022-27976-8.

Doria, Enrico; Galleschi, Luciano; Calucci, Lucia; Pinzino, Calogero; Pilu, Roberto; Cassani, Elena; Nielsen, Erik (2009): Phytic acid prevents oxidative stress in seeds: evidence from a maize (*Zea mays* L.) low phytic acid mutant. In *J Exp Bot* 60 (3), pp. 967–978. DOI: 10.1093/jxb/ern345.

Doubled haploid technology in maize breeding: theory and practice (2012). With assistance of B. M. Prasanna, Vijay Chaikam, George Mahuku. Mexico: CIMMYT.

Duvick, Donald N. (2001): Biotechnology in the 1930s: the development of hybrid maize. In *Nat Rev Genet* 2 (1), pp. 69–74. DOI: 10.1038/35047587.

Duvick, Donald N. (2005): The Contribution of Breeding to Yield Advances in maize (*Zea mays* L.) (86). Available online at https://www.sciencedirect.com/science/article/pii/S006521130586002X.

Elgorashi Bakhite, Mohammed A.; Sithole, Nkanyiso J.; Magwaza, Lembe S.; Odindo, Alfred O.; Derera, John (2021): Comparison of seed germination and vigour in low and high phytic acid maize synthetic populations and commercially available hybrids. In *South African Journal of Plant and Soil* 38 (4), pp. 285–293. DOI: 10.1080/02571862.2021.1907465.

European Commission (Ed.) (2020): Farm to Fork Strategy. For a fair, healthy and environmentally-friendly food system. #EUGreenDeal. European Commission. Available online at https://ec.europa.eu/food/system/files/2020-05/f2f_action-plan_2020_strategy-info_en.pdf, checked on 1/26/2022.

FAOSTAT (2019): Data > Inputs. Edited by Food and Agriculture Organization of the United Nations. Available online at http://www.fao.org/faostat/en/#data/RFN, checked on 3/29/2022.

Federal Ministry of Food and Agriculture (2017): Verordnung über die Anwendung von Düngemitteln, Bodenhilfsstoffen, Kultursubstraten und Pflanzenhilfsmitteln nach den Grundsätzen der guten fachlichen Praxis beim Düngen (Düngeverordnung - DüV) [Ordinance on the Application of Fertilizers, Soil Additives, Cultivation Substrates and Plant Auxiliaries in Accordance with the Principles of Good Fertilizing Practice (Fertilizer Ordinance - DüV)]. DüV, pp. 1–46. Available online at http://www.gesetze-im-internet.de/d_v_2017/D%C3%BCV.pdf.

Frank Stephano, Mabagala; Geng, Yuhui; Cao, Guojun; Wang, Lichun; Meng, Wang; Meiling, Zhang (2021): Effect of Silicon Fertilizer and Straw Return on the Maize Yield and Phosphorus Efficiency in Northeast China. In *Communications in Soil Science and Plant Analysis* 52 (2), pp. 116–127. DOI: 10.1080/00103624.2020.1854284.

Frascaroli, Elisabetta; Revilla, Pedro (2018): Genomics of Cold Tolerance in Maize. In : The Maize Genome: Springer, Cham, pp. 287–303. Available online at https://link.springer.com/chapter/10.1007/978-3-319-97427-9_17.

Frascaroli, Elisabetta; Schrag, Tobias A.; Melchinger, Albrecht E. (2013): Genetic diversity analysis of elite European maize (*Zea mays* L.) inbred lines using AFLP, SSR, and SNP markers reveals ascertainment bias for a subset of SNPs. In *Theor Appl Genet* 126 (1), pp. 133–141. DOI: 10.1007/s00122-012-1968-6.

Fuchs, Richard; Brown, Calum; Rounsevell, Mark (2020): Europe's Green Deal offshores environmental damage to other nations. In *Nature* 586 (7831), pp. 671–673. DOI: 10.1038/d41586-020-02991-1.

Galiba, Gábor; Vágújfalvi, Attila; Li, Chengxia; Soltész, Alexandra; Dubcovsky, Jorge (2009): Regulatory genes involved in the determination of frost tolerance in temperate cereals. In *Plant Science* 176 (1), pp. 12–19. DOI: 10.1016/j.plantsci.2008.09.016.

Gasura, Edmore; Setimela, Peter; Edema, Richard; Gibson, Paul T.; Okori, Patrick; Tarekegne, Amsal (2013): Exploiting Grain-Filling Rate and Effective Grain-Filling Duration to Improve Grain Yield of Early-Maturing Maize. In *Crop Sci.* 53 (6), pp. 2295–2303. DOI: 10.2135/cropsci2013.01.0032.

Geiger, H. H.; Gordillo, G. A. (2009): Doubled Haploids in Hybrid maize breeding. In *Maydica* 54 (4), pp. 485–499.

German Maize Committee (2022): Maisanbaufläche Deutschland in ha [Maize acreage Germany in ha]. 2020 (endgültig) und 2021 (endgültig) nach Bundesländern und Nutzungsrichtung in ha. Edited by D.M.K. Statistisches Bundesamt. Available online at https://www.maiskomitee.de/web/upload/pdf/statistik/dateien_pdf/Maisanbauflaechen_D_je_BL_in_ha_2020-2021_endgueltig_20220207.pdf.

Gordon, W. B.; Fjell, D. L.; Whitney, D. A. (1997): Corn Hybrid Response to Starter Fertilizer in a No-Tillage, Dryland Environment. In *J. Prod. Agric* 10 (3), pp. 347–404.

Görlach, Bruno Maximilian; Mühling, Karl Hermann (2021): Phosphate foliar application increases biomass and P concentration in P deficient maize. In *J. Plant Nutr. Soil Sci.* 184 (3), pp. 360–370. DOI: 10.1002/jpln.202000460.

Hallauer, A. R. (1999): Temperate Maize and Heterosis. In J. G. Coors, Shivaji Pandey, Arnel R. Hallauer, Delbert C. Hess, Maarten van Ginkel, Kendall R. Lamkey et al. (Eds.): Genetics and exploitation of heterosis in crops. Madison, Wisconsin, USA: American Society of Agronomy Inc (ASA, CSSA, and SSSA Books), pp. 353–361.

Hallauer, A. R.; Russell, Wilbert; Lamkey, Kendall; Lamkey, K. R. (1988): Corn Breeding. Available online at https://dr.lib.iastate.edu/entities/publication/87184df4-8573-41eb-9f66-26c2e3051cc6.

Hallauer, Arnel; Filho, J. B. Miranda; Carena, Marcelo J. (2010): Quantitative genetics in maize breeding. 3rd ed. New York: Springer (Handbook of plant breeding, 7290).

Hertzberger, Allan J.; Cusick, Roland D.; Margenot, Andrew J. (2021): Maize and soybean response to phosphorus fertilization with blends of struvite and monoammonium phosphate. In *Plant Soil* 461 (1-2), pp. 547–563. DOI: 10.1007/s11104-021-04830-2.

Hinsinger, Philippe (2001): Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. In *Plant Soil* 237 (2), pp. 173–195. DOI: 10.1023/A:1013351617532.

Hölker, Armin C.; Mayer, Manfred; Presterl, Thomas; Bolduan, Therese; Bauer, Eva; Ordas, Bernardo et al. (2019a): European maize landraces made accessible for plant breeding and genome-based studies. In *Theor Appl Genet* 132 (12), pp. 3333–3345. DOI: 10.1007/s00122-019-03428-8.

Hölker, Armin C.; Schipprack, Wolfgang; Utz, H. Friedrich; Molenaar, Willem S.; Melchinger, Albrecht E. (2019b): Progress for testcross performance within the flint heterotic pool of a public maize breeding program since the onset of hybrid breeding. In *Euphytica* 215 (3). DOI: 10.1007/s10681-019-2370-0.

Jähne, Felix; Hahn, Volker; Würschum, Tobias; Leiser, Willmar L. (2020): Speed breeding short-day crops by LED-controlled light schemes. In *Theor Appl Genet* 133 (8), pp. 2335–2342. DOI: 10.1007/s00122-020-03601-4.

Jing, J.; Zhang, F.; Rengel, Z.; Shen, J. (2012): Localized fertilization with P plus N elicits an ammonium-dependent enhancement of maize root growth and nutrient uptake. In *Field Crops Res* 133, pp. 176–185. DOI: 10.1016/j.fcr.2012.04.009.

Joshi, Sunendra R.; Morris, James W.; Tfaily, Malak M.; Young, Robert P.; McNear, David H. (2021): Low soil phosphorus availability triggers maize growth stage specific rhizosphere processes leading to mineralization of organic P. In *Plant Soil* 459 (1-2), pp. 423–440. DOI: 10.1007/s11104-020-04774-z.

Kanno, Satomi; Cuyas, Laura; Javot, Hélène; Bligny, Richard; Gout, Elisabeth; Dartevelle, Thibault et al. (2016): Performance and Limitations of Phosphate Quantification: Guidelines for Plant Biologists. In *Plant & cell physiology* 57 (4), pp. 690–706. DOI: 10.1093/pcp/pcv208.

Kenea, Workneh Bekere; Reidsma, Pytrik; Descheemaeker, Katrien; Rurinda, Jairos; Balemi, Tesfaye; van Ittersum, Martin K. (2021): Variability in yield responses, physiological use efficiencies and recovery fractions of fertilizer use in maize in Ethiopia. In *European Journal of Agronomy* 124, p. 126228. DOI: 10.1016/j.eja.2020.126228.

Komarek, Adam M.; Drogue, Sophie; Chenoune, Roza; Hawkins, James; Msangi, Siwa; Belhouchette, Hatem; Flichman, Guillermo (2017): Agricultural household effects of fertilizer price changes for smallholder farmers in central Malawi. In *Agricultural Systems* 154, pp. 168–178. DOI: 10.1016/j.agsy.2017.03.016.

Kostic, Ljiljana; Nikolic, Nina; Bosnic, Dragana; Samardzic, Jelena; Nikolic, Miroslav (2017): Silicon increases phosphorus (P) uptake by wheat under low P acid soil conditions. In *Plant and Soil* 419 (1-2), pp. 447–455. DOI: 10.1007/s11104-017-3364-0.

Lambers, Hans; Wright, Ian J.; Guilherme Pereira, Caio; Bellingham, Peter J.; Bentley, Lisa Patrick; Boonman, Alex et al. (2021): Leaf manganese concentrations as a tool to assess belowground plant functioning in phosphorus-impoverished environments. In *Plant Soil* 461 (1-2), pp. 43–61. DOI: 10.1007/s11104-020-04690-2.

Lane, Holly M.; Murray, Seth C.; Montesinos-López, Osval A.; Montesinos-López, Abelardo; Crossa, José; Rooney, David K. et al. (2020): Phenomic selection and prediction of maize grain yield from near-infrared reflectance spectroscopy of kernels. In *Plant phenome j.* 3 (1), e0117737. DOI: 10.1002/ppj2.20002.

Leiser, Willmar L. (2014): Sorghum Breeding Strategies for Phosphorus-Limited Environments in Western Sorghum Breeding Strategies for Phosphorus-Limited Environments in Western Africa: From Field to Genome Level. Edited by University of Hohenheim. Available online at http://opus.uni-hohenheim.de/volltexte/2014/1012/.

Leiser, Willmar L.; Olatoye, Marcus O.; Rattunde, H. Frederick W.; Neumann, Günter; Weltzien, Eva; Haussmann, Bettina I. G. (2016): No need to breed for enhanced colonization by arbuscular mycorrhizal fungi to improve low-P adaptation of West African sorghums. In *Plant Soil* 401 (1-2), pp. 51–64. DOI: 10.1007/s11104-015-2437-1.

Leiser, Willmar L.; Rattunde, H. Frederick W.; Piepho, Hans-Peter; Weltzien, Eva; Diallo, Abdoulaye; Melchinger, Albrecht E. et al. (2012): Selection Strategy for Sorghum Targeting Phosphorus-limited Environments in West Africa: Analysis of Multi-environment Experiments. In *Crop Science* 52 (6), pp. 2517–2527. DOI: 10.2135/cropsci2012.02.0139.

Leiser, Willmar L.; Rattunde, H. Frederick W.; Piepho, Hans-Peter; Weltzien, Eva; Diallo, Abdoulaye; Toure, Abocar; Haussmann, Bettina I.G. (2015): Phosphorous Efficiency and Tolerance Traits for Selection of Sorghum for Performance in Phosphorous-Limited Environments. In *Crop Science* 55 (3), pp. 1152–1162. DOI: 10.2135/cropsci2014.05.0392.

Leiser, Willmar L.; Rattunde, H. Frederick W.; Weltzien, Eva; Haussmann, Bettina I. G. (2014a): Phosphorus uptake and use efficiency of diverse West and Central African sorghum genotypes under field conditions in Mali. In *Plant Soil* 377 (1-2), pp. 383–394. DOI: 10.1007/s11104-013-1978-4.

Leiser, Willmar L.; Rattunde, Henry Frederick W.; Weltzien, Eva; Cisse, Ndiaga; Abdou, Magagi; Diallo, Abdoulaye et al. (2014b): Two in one sweep: aluminum tolerance and grain yield in P-limited soils are associated to the same genomic region in West African sorghum. In *BMC plant biology* 14, p. 206. DOI: 10.1186/s12870-014-0206-6.

Li, Dongdong; Chen, Zhe; Wang, Meng; Leiser, Willmar L.; Weiß, Thea Mi; Zhao, Zheng et al. (2021a): Dissecting the phenotypic response of maize to low phosphorus soils by field screening of a large diversity panel. In *Euphytica* 217 (1). DOI: 10.1007/s10681-020-02727-2.

Li, Dongdong; Wang, Haoying; Wang, Meng; Li, Guoliang; Chen, Zhe; Leiser, Willmar L. et al. (2021b): Genetic Dissection of Phosphorus Use Efficiency in a Maize Association Population under Two P Levels in the Field. In *International journal of molecular sciences* 22 (17), p. 9311. DOI: 10.3390/ijms22179311.

Li, Dongdong; Wang, Meng; Kuang, Xianyan; Liu, Wenxin (2019a): Genetic study and molecular breeding for high phosphorus use efficiency in maize. In *Front. Agr. Sci. Eng.* 6 (4), p. 366. DOI: 10.15302/J-FASE-2019278.

Li, Mo; Wiedmann, Thomas; Hadjikakou, Michalis (2019b): Towards meaningful consumption-based planetary boundary indicators: The phosphorus exceedance footprint. In *Global Environmental Change* 54, pp. 227–238. DOI: 10.1016/j.gloenvcha.2018.12.005.

Liebig, Justus von (1841): Die organische Chemie in ihrer Anwendung auf Agricultur und Physiologie [Organic chemistry in its application to agriculture and physiology]: Vieweg. Available online at https://www.digitale-sammlungen.de/de/view/bsb10707847?page=178,179&q=phos, checked on 3/29/2022.

Lim, Sooyeon; Yi, Gibum (2019): Investigating seed mineral composition in Korean landrace maize (*Zea mays* L.) and its kernel texture specificity. In *J. Integr. Agric* 18 (9), pp. 1996–2005. DOI: 10.1016/S2095-3119(18)62055-6.

Lipman, E.; Ellis, R. H.; Gass, T. (Eds.) (1997): Maize genetic resources in Europe. Report of a Workshop, 28-29 May 1996, Rome, Italy. Rome: International Plant Genetic Resources Institute.

Liu, Fang; Xu, Yunjian; Jiang, Huanhuan; Jiang, Chaosheng; Du, Yibin; Gong, Cheng et al. (2016): Systematic Identification, Evolution and Expression Analysis of the Zea mays PHT1 Gene Family Reveals Several New Members Involved in Root Colonization by Arbuscular Mycorrhizal Fungi. In *International journal of molecular sciences* 17 (6). DOI: 10.3390/ijms17060930.

Liu, Haijun; Wang, Xiaqing; Warburton, Marilyn L.; Wen, Weiwei; Jin, Minliang; Deng, Min et al. (2015): Genomic, Transcriptomic, and Phenomic Variation Reveals the Complex Adaptation of Modern Maize Breeding. In *Molecular plant* 8 (6), pp. 871–884. DOI: 10.1016/j.molp.2015.01.016.

Liu, Wenxin; Gowda, Manje; Steinhoff, Jana; Maurer, Hans Peter; Würschum, Tobias; Longin, Carl Friedrich Horst et al. (2011): Association mapping in an elite maize breeding population. In *Theor Appl Genet* 123 (5), pp. 847–858. DOI: 10.1007/s00122-011-1631-7.

Liu, Wenxin; Maurer, Hans Peter; Li, Guoliang; Tucker, Matthew R.; Gowda, Manje; Weissmann, Elmar A. et al. (2014): Genetic architecture of winter hardiness and frost tolerance in triticale. In *PLOS ONE* 9 (6), e99848. DOI: 10.1371/journal.pone.0099848.

Liu, Yi; Villalba, Gara; Ayres, Robert U.; Schroder, Hans (2008): Global Phosphorus Flows and Environmental Impacts from a Consumption Perspective. In *J Ind Ecol* 12 (2), pp. 229–247. DOI: 10.1111/j.1530-9290.2008.00025.x.

Liu, Zhigang; Gao, Kun; Shan, Shengchen; Gu, Riling; Wang, Zhangkui; Craft, Eric J. et al. (2017): Comparative Analysis of Root Traits and the Associated QTLs for Maize Seedlings Grown in Paper Roll, Hydroponics and Vermiculite Culture System. In *Front. Plant Sci.* 8, p. 436. DOI: 10.3389/fpls.2017.00436.

Lu, Xianju; Wang, Jinglu; Wang, Yongjian; Wen, Weiliang; Zhang, Ying; Du, Jianjun et al. (2020): Genome-Wide Association Study of Maize Aboveground Dry Matter Accumulation at Seedling Stage. In *Frontiers in genetics* 11, p. 571236. DOI: 10.3389/fgene.2020.571236.

Ludewig, Uwe; Yuan, Lixing; Neumann, Günter (2019): Improving the efficiency and effectiveness of global phosphorus use: focus on root and rhizosphere levels in the agronomic system. In *Front. Agr. Sci. Eng.* 6 (4), p. 357. DOI: 10.15302/J-FASE-2019275.

Lun, Fei; Sardans, Jordi; Sun, Danfeng; Xiao, Xiao; Liu, Ming; Li, Zhuo et al. (2021): Influences of international agricultural trade on the global phosphorus cycle and its associated issues. In *Global Environmental Change* 69, p. 102282. DOI: 10.1016/j.gloenvcha.2021.102282.

Lynch, Jonathan P. (2007): Rhizoeconomics: The Roots of Shoot Growth Limitations. In *horts* 42 (5), pp. 1107–1109. DOI: 10.21273/HORTSCI.42.5.1107.

Ma, Xiaomin; Li, Xuelian; Ludewig, Uwe (2021): Arbuscular mycorrhizal colonization outcompetes root hairs in maize under low phosphorus availability. In *Annals of botany* 127 (1), pp. 155–166. DOI: 10.1093/aob/mcaa159.

Magalhaes, Jurandir V.; Liu, Jiping; Guimarães, Claudia T.; Lana, Ubiraci G. P.; Alves, Vera M. C.; Wang, Yi-Hong et al. (2007): A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. In *Nature genetics* 39 (9), pp. 1156–1161. DOI: 10.1038/ng2074.

Magalhaes, Jurandir V.; Sousa, Sylvia M. de; Guimaraes, Claudia T.; Kochian, Leon V. (2018): Plant Macronutrient Use Efficiency Molecular and Genomic Perspectives in Crop. Chapter 7: The role of root morphology and architecture in phosphorus acquisition:

physiological, genetic, and molecular basis. With assistance of Mohammad Anwar Hossain, Takehiro Kamiya, David J. Burritt, Lam-Son Phan Tran, Toru Fujiwara: Elsevier.

Mascagni Jr., H. J.; Boquet, D. J. (1996): Starter Fertilizer and Planting Date Effects on Corn Rotated with Cotton. In *J. Agron* (88), pp. 975–982.

Mayer, Manfred; Hölker, Armin C.; González-Segovia, Eric; Bauer, Eva; Presterl, Thomas; Ouzunova, Milena et al. (2020): Discovery of beneficial haplotypes for complex traits in maize landraces. In *Nature communications* 11 (1), p. 4954. DOI: 10.1038/s41467-020-18683-3.

Melchinger, Albrecht E.; Schopp, Pascal; Müller, Dominik; Schrag, Tobias A.; Bauer, Eva; Unterseer, Sandra et al. (2017): Safeguarding Our Genetic Resources with Libraries of Doubled-Haploid Lines. In *Genetics* 206 (3), pp. 1611–1619. DOI: 10.1534/genetics.115.186205.

Messiga, Aimé J.; Lam, Camellia; Li, Yunkun; Kidd, Steven; Yu, Shaobing; Bineng, Carine (2020): Combined Starter Phosphorus and Manure Applications on Silage Corn Yield and Phosphorus Uptake in Southern BC. In *Front. Earth Sci.* 8. DOI: 10.3389/feart.2020.00088.

Moradtalab, Narges; Weinmann, Markus; Walker, Frank; Höglinger, Birgit; Ludewig, Uwe; Neumann, Guenter (2018): Silicon Improves Chilling Tolerance During Early Growth of Maize by Effects on Micronutrient Homeostasis and Hormonal Balances. In *Frontiers in plant science* 9, p. 420. DOI: 10.3389/fpls.2018.00420.

Mpanga, Isaac Kwadwo; Nkebiwe, Peteh Mehdi; Kuhlmann, Mira; Cozzolino, Vincenza; Piccolo, Alessandro; Geistlinger, Jörg et al. (2019): The Form of N Supply Determines Plant Growth Promotion by P-Solubilizing Microorganisms in Maize. In *Microorganisms* 7 (2), p. 38. DOI: 10.3390/microorganisms7020038.

Nagy, R.; Vasconcelos, M. J. V.; Zhao, S.; McElver, J.; Bruce, W.; Amrhein, N. et al. (2006): Differential regulation of five Pht1 phosphate transporters from maize (*Zea mays* L.). In *Plant biology (Stuttgart, Germany)* 8 (2), pp. 186–197. DOI: 10.1055/s-2005-873052.

Nkebiwe, Peteh Mehdi; Weinmann, Markus; Bar-Tal, Asher; Müller, Torsten (2016): Fertilizer placement to improve crop nutrient acquisition and yield: A review and meta-analysis. In *Field Crops Research* 196, pp. 389–401. DOI: 10.1016/j.fcr.2016.07.018.

Nussaume, Laurent; Kanno, Satomi; Javot, Hélène; Marin, Elena; Pochon, Nathalie; Ayadi, Amal et al. (2011): Phosphate Import in Plants: Focus on the PHT1 Transporters. In *Front. Plant Sci.* 2, p. 83. DOI: 10.3389/fpls.2011.00083.

Palmer, Christine M.; Guerinot, Mary Lou (2009): Facing the challenges of Cu, Fe and Zn homeostasis in plants. In *Nature chemical biology* 5 (5), pp. 333–340. DOI: 10.1038/nchembio.166.

Parentoni, Sidney Netto; Souza Júnior, C. L. de (2008): Phosphorus acquisition and internal utilization efficiency in tropical maize genotypes. In *Pesq. agropec. bras., Brasília* 43 (7), pp. 893–901.

Pedersen, Ingeborg F.; Sørensen, Peter; Rasmussen, Jim; Withers, Paul J. A.; Rubaek, Gitte Holton (2019): Fertilizer ammonium: nitrate ratios determine phosphorus uptake by young maize plants. In *J. Soil Sci. Plant Nutr* 182 (4), pp. 541–551. DOI: 10.1002/jpln.201800553.

Pedersen, Ingeborg Frøsig; Christensen, Julie Therese; Sørensen, Peter; Christensen, Bent T.; Holton Rubæk, Gitte (2022): Early plant height: A defining factor for yields of silage maize with contrasting phosphorus supply. In *Soil Use and Management* 38 (1), pp. 537–548. DOI: 10.1111/sum.12697.

Peter, Roland; Eschholz, Tobias W.; Stamp, Peter; Liedgens, Markus (2009): Swiss Flint maize landraces—A rich pool of variability for early vigour in cool environments. In *Field Crops Research* 110 (2), pp. 157–166. DOI: 10.1016/j.fcr.2008.07.015.

Raboy, Victor (2002): Progress in breeding low phytate crops. In *J Nutr* 132 (3), 503S-505S. DOI: 10.1093/jn/132.3.503S.

Raboy, Victor; Young, Kevin A.; Dorsch, John A.; Cook, Allen (2001): Genetics and breeding of seed phosphorus and phytic acid. In *Journal of Plant Physiology* 158 (4), pp. 489–497. DOI: 10.1078/0176-1617-00361.

Ragi, Shridhar; Muthusamy, Vignesh; Zunjare, Rajkumar U.; Bhatt, Vinay; Katral, Ashvinkumar; Abhijith, Krishnan P. et al. (2022): Genetic variation for grain phytate and molecular characterization of *low phytic acid-2* (*lpa2*) gene-based maize (*Zea mays*) inbreds. In *Plant Breeding*. DOI: 10.1111/pbr.13006.

Ran, F. Ann; Hsu, Patrick D.; Wright, Jason; Agarwala, Vineeta; Scott, David A.; Zhang, Feng (2013): Genome engineering using the CRISPR-Cas9 system. In *Nature protocols* 8 (11), pp. 2281–2308. DOI: 10.1038/nprot.2013.143.

Rebourg, C.; Chastanet, M.; Gouesnard, B.; Welcker, C.; Dubreuil, P.; Charcosset, Alain (2003): Maize introduction into Europe: the history reviewed in the light of molecular data. In *Theor Appl Genet* 106 (5), pp. 895–903. DOI: 10.1007/s00122-002-1140-9.

Revilla, P.; Malvar, R. A.; Cartea, M. E.; Butrón, A.; Ordás, A. (2000): Inheritance of Cold Tolerance at Emergence and during Early Season Growth in Maize. In *Crop Sci.* 40 (6), pp. 1579–1585. DOI: 10.2135/cropsci2000.4061579x.

Rincent, Renaud; Charpentier, Jean-Paul; Faivre-Rampant, Patricia; Paux, Etienne; Le Gouis, Jacques; Bastien, Catherine; Segura, Vincent (2018): Phenomic Selection Is a Low-Cost and High-Throughput Method Based on Indirect Predictions: Proof of Concept on Wheat and Poplar. In *G3 (Bethesda, Md.)* 8 (12), pp. 3961–3972. DOI: 10.1534/g3.118.200760.

Röber, F. K.; Gordillo, G. A.; Geiger, H. H. (2005): In vivo haploid induction in maize. Performance of new inducers and significance of doubled haploid lines in hybrid breeding [*Zea mays* L.]. In *Maydica (Italy)*. Available online at https://agris.fao.org/agris-search/search.do?recordID=IT2006602235.

Robert, Pauline; Auzanneau, Jérôme; Goudemand, Ellen; Oury, François-Xavier; Rolland, Bernard; Heumez, Emmanuel et al. (2022): Phenomic selection in wheat breeding: identification and optimisation of factors influencing prediction accuracy and comparison to genomic selection. In *Theor Appl Genet* 135 (3), pp. 895–914. DOI: 10.1007/s00122-021-04005-8.

Rose, Terry J.; Mori, Asako; Julia, Cecile C.; Wissuwa, Matthias (2016): Screening for internal phosphorus utilisation efficiency: comparison of genotypes at equal shoot P content is critical. In *Plant Soil* 401 (1-2), pp. 79–91. DOI: 10.1007/s11104-015-2565-7.

Rose, Terry J.; Wissuwa, Matthias (2012): Rethinking Internal Phosphorus Utilization Efficiency. In : Advances in Agronomy, vol. 116: Elsevier (Advances in Agronomy), pp. 185–217.

Saboor, Abdul; Ali, Muhammad Arif; Hussain, Shabir; El Enshasy, Hesham A.; Hussain, Sajjad; Ahmed, Niaz et al. (2021): Zinc nutrition and arbuscular mycorrhizal symbiosis effects on maize (*Zea mays* L.) growth and productivity. In *Saudi Journal of Biological Sciences* 28 (11), pp. 6339–6351. DOI: 10.1016/j.sjbs.2021.06.096.

Shakiba, Ehsan; Edwards, Jeremy D.; Jodari, Farman; Duke, Sara E.; Baldo, Angela M.; Korniliev, Pavel et al. (2017): Genetic architecture of cold tolerance in rice (*Oryza sativa*) determined through high resolution genome-wide analysis. In *PLOS ONE* 12 (3), e0172133. DOI: 10.1371/journal.pone.0172133.

Shull, G. H. (1909): A Pure-Line Method in Corn Breeding. In *J Hered* os-5 (1), pp. 51–58. DOI: 10.1093/jhered/os-5.1.51.

Smith, V. H. (2009): Eutrophication. In Gene E. Likens (Ed.): Encyclopedia of inland waters. Amsterdam: ScienceDirect, pp. 61–73. Available online at https://www.sciencedirect.com/science/article/pii/B9780123706263002349.

Soares, José C.; Zimmermann, Lars; Zendonadi dos Santos, Nicolas; Muller, Onno; Pintado, Manuela; Vasconcelos, Marta W. (2021): Genotypic variation in the response of soybean to elevated CO 2. In *Plant-Environment Interactions* 2 (6), pp. 263–276. DOI: 10.1002/pei3.10065.

Steinhoff, Jana; Liu, Wenxin; Maurer, Hans Peter; Würschum, Tobias; C. Friedrich, H. Longin; Ranc, Nicolas; Reif, Jochen C. (2012): Exploitation of Elite Maize (*Zea mays* L.) Germplasm across Maturity Zones. In *Crop Sci.* 52 (4), pp. 1534–1542. DOI: 10.2135/cropsci2011.10.0533.

Sun, Yankun; Xu, Jiaqi; Miao, Xiangyang; Lin, Xuesong; Liu, Wanzhen; Ren, Hongyu (2021): Effects of exogenous silicon on maize seed germination and seedling growth. In *Sci Rep* 11 (1), p. 1014. DOI: 10.1038/s41598-020-79723-y.

Teare, I. D.; Wright, D. L. (1990): Corn Hybrid-Starter Fertilizer Interaction for Yield and Lodging. In *Crop Sci* (30), pp. 1298–1303.

Tenaillon, Maud Irène; Charcosset, Alain (2011): A European perspective on maize history. In *Comptes Rendus Biologies* 334 (3), pp. 221–228. DOI: 10.1016/j.crvi.2010.12.015.

Unterseer, Sandra; Pophaly, Saurabh D.; Peis, Regina; Westermeier, Peter; Mayer, Manfred; Seidel, Michael A. et al. (2016): A comprehensive study of the genomic differentiation between temperate Dent and Flint maize. In *Genome Biol* 17 (1), p. 137. DOI: 10.1186/s13059-016-1009-x.

van Vuuren, D. P.; Bouwman, A. F.; Beusen, A.H.W. (2010): Phosphorus demand for the 1970–2100 period: A scenario analysis of resource depletion. In *Global Environmental Change* 20 (3), pp. 428–439. DOI: 10.1016/j.gloenvcha.2010.04.004.

van Wesenbeeck, C.F.A.; Keyzer, M. A.; van Veen, W.C.M.; Qiu, H. (2021): Can China's overuse of fertilizer be reduced without threatening food security and farm incomes? In *Agricultural Systems* 190, p. 103093. DOI: 10.1016/j.agsy.2021.103093.

VandenBygaart, A. J.; Geng, X.; He, J. (2021): Spatial decoupling of legacy phosphorus in cropland: Soil erosion and deposition as a mechanism for storage. In *Soil and Tillage Research* 211, p. 105050. DOI: 10.1016/j.still.2021.105050.

Victor Roch, Gurusunathan; Maharajan, Theivanayagam; Ceasar, Stanislaus Antony; Ignacimuthu, Savarimuthu (2019): The Role of *PHT1* Family Transporters in the Acquisition and Redistribution of Phosphorus in Plants. In *Critical Reviews in Plant Sciences* 38 (3), pp. 171–198. DOI: 10.1080/07352689.2019.1645402.

Vitousek, P. M.; Naylor, R.; Crews, T.; David, M. B.; Drinkwater, L. E.; Holland, E. et al. (2009): Agriculture. Nutrient imbalances in agricultural development. In *Science* 324 (5934), pp. 1519–1520. DOI: 10.1126/science.1170261.

Vogel, Christian; Sekine, Ryo; Huang, Jianyin; Steckenmesser, Daniel; Steffens, Diedrich; Huthwelker, Thomas et al. (2020): Effects of a nitrification inhibitor on nitrogen species in the soil and the yield and phosphorus uptake of maize. In *The Science of the total environment* 715, p. 136895. DOI: 10.1016/j.scitotenv.2020.136895.

Wang, Fanmiao; King, James Douglas Morrison; Rose, Terry; Kretzschmar, Tobias; Wissuwa, Matthias (2017): Can natural variation in grain P concentrations be exploited in rice breeding to lower fertilizer requirements? In *PloS one* 12 (6), e0179484. DOI: 10.1371/journal.pone.0179484.

Weih, Martin; Liu, Hui; Colombi, Tino; Keller, Thomas; Jäck, Ortrud; Vallenback, Pernilla; Westerbergh, Anna (2021): Evidence for magnesium-phosphorus synergism and co-limitation of grain yield in wheat agriculture. In *Sci Rep* 11 (1), p. 9012. DOI: 10.1038/s41598-021-88588-8.

Weiß, Thea Mi; Leiser, Willmar L.; Reineke, Alice-J; Li, Dongdong; Liu, Wenxin; Hahn, Volker; Würschum, Tobias (2021): Optimizing the P balance: How do modern maize hybrids react to different starter fertilizers? In *PloS one* 16 (4), e0250496. DOI: 10.1371/journal.pone.0250496.

Weiß, Thea Mi; Li, Dongdong; Roller, Sandra; Liu, Wenxin; Hahn, Volker; Leiser, Willmar L.; Würschum, Tobias (unpublished): How can we breed for phosphate efficiency in maize (*Zea mays* L.)? In *[manuscript submitted]*.

Weiß, Thea Mi; Zhu, Xintian; Leiser, Willmar L.; Li, Dongdong; Liu, Wenxin; Schipprack, Wolfgang et al. (2022): Unraveling the potential of phenomic selection within and among diverse breeding material of maize (*Zea mays* L.). In *G3 (Bethesda, Md.)*. DOI: 10.1093/g3journal/jkab445.

Wilde, K.; Burger, H.; Prigge, V.; Presterl, T.; Schmidt, W.; Ouzunova, M.; Geiger, H. H. (2010): Testcross performance of doubled-haploid lines developed from European flint maize landraces. In *Plant Breeding* 129 (2), pp. 181–185. DOI: 10.1111/j.1439-0523.2009.01677.x.

Wissuwa, Matthias; Ae, Noriharu (2001): Genotypic variation for tolerance to phosphorus defciency in rice and the potential for its exploitation in rice improvement. In *Plant Breeding* (120), pp. 43–48.

Withers, Paul J. A.; Forber, Kirsty G.; Lyon, Christopher; Rothwell, Shane; Doody, Donnacha G.; Jarvie, Helen P. et al. (2020): Towards resolving the phosphorus chaos created by food systems. In *Ambio* 49 (5), pp. 1076–1089. DOI: 10.1007/s13280-019-01255-1.

Wu, Bin; Ren, Wei; Zhao, Longfei; Li, Qiang; Sun, Jiazheng; Chen, Fanjun; Pan, Qingchun (2022): Genome-Wide Association Study of Root System Architecture in Maize. In *Genes* 13 (2). DOI: 10.3390/genes13020181.

Wu, Huijun; Huang, Zhouman; Gao, Liangmin; Li, Haoran; Yang, Tao; Yang, Jinxiang; Li, Xiaolong (2021): Understanding Farmers' Behaviors and Driving Factors Toward Phosphorus Security of Crop Farming in Chaohu Watershed, China. In *Pol. J. Environ. Stud.* 30 (2), pp. 1865–1879. DOI: 10.15244/pjoes/126489.

Würschum, Tobias; Weiß, Thea Mi; Renner, Juliane; Friedrich Utz, H.; Gierl, Alfons; Jonczyk, Rafal et al. (2022): High-resolution association mapping with libraries of immortalized lines from ancestral landraces. In *TAG. Theoretical and applied genetics* (135), pp. 243–256. DOI: 10.1007/s00122-021-03963-3.

Xin, Xiuli; Qin, Shengwu; Zhang, Jiabao; Zhu, Anning; Yang, Wenliang; Zhang, Xianfeng (2017): Yield, phosphorus use efficiency and balance response to substituting long-term chemical fertilizer use with organic manure in a wheat-maize system. In *Field Crops Research* 208, pp. 27–33. DOI: 10.1016/j.fcr.2017.03.011.

Yan, Xiaojun; Chen, Xiaohui; Ma, Changcheng; Cai, Yuanyang; Cui, Zhenling; Chen, Xinping et al. (2021): What are the key factors affecting maize yield response to and agronomic efficiency of phosphorus fertilizer in China? In *Field Crops Research* 270, p. 108221. DOI: 10.1016/j.fcr.2021.108221.

Yi, Q.; Álvarez-Iglesias, L.; Malvar, R. A.; Romay, M. C.; Revilla, Pedro (2021): A worldwide maize panel revealed new genetic variation for cold tolerance. In *Theor Appl Genet* 134 (4), pp. 1083–1094. DOI: 10.1007/s00122-020-03753-3.

Yu, Xing; Keitel, Claudia; Dijkstra, Feike A. (2021): Global analysis of phosphorus fertilizer use efficiency in cereal crops. In *Global Food Security* 29, p. 100545. DOI: 10.1016/j.gfs.2021.100545.

Zhang, Haohao; Yin, Lilin; Wang, Meiyue; Yuan, Xiaohui; Liu, Xiaolei (2019): Factors Affecting the Accuracy of Genomic Selection for Agricultural Economic Traits in Maize, Cattle, and Pig Populations. In *Front. Genet.* 10, p. 189. DOI: 10.3389/fgene.2019.00189.

Zhang, Hongwei; Uddin, Mohammed Shalim; Zou, Cheng; Xie, Chuanxiao; Xu, Yunbi; Li, Wen-Xue (2014): Meta-analysis and candidate gene mining of low-phosphorus tolerance in maize. In *Journal of integrative plant biology* 56 (3), pp. 262–270. DOI: 10.1111/jipb.12168.

Zhang, Jie; Beusen, Arthur H. W.; van Apeldoorn, Dirk F.; Mogollón, José M.; Yu, Chaoqing; Bouwman, Alexander F. (2017): Spatiotemporal dynamics of soil phosphorus and crop uptake in global cropland during the 20th century. In *Biogeosciences* 14 (8), pp. 2055–2068. DOI: 10.5194/bg-14-2055-2017.

Zhang, Junhong; Lin, Yan; Wu, Fangmin; Zhang, Yuting; Cheng, Longjun; Huang, Menghui; Tong, Zaikang (2021a): Profiling of MicroRNAs and Their Targets in Roots and Shoots Reveals a Potential MiRNA-Mediated Interaction Network in Response to Phosphate Deficiency in the Forestry Tree *Betula luminifera*. In *Frontiers in genetics* 12, p. 552454. DOI: 10.3389/fgene.2021.552454.

Zhang, Wei; Zhang, Wushuai; Wang, Xiaozhong; Liu, Dunyi; Zou, Chunqin; Chen, Xinping (2021b): Quantitative evaluation of the grain zinc in cereal crops caused by phosphorus fertilization. A meta-analysis. In *Agron. Sustain. Dev.* 41 (1), pp. 1–12. DOI: 10.1007/s13593-020-00661-0.

Zhang, Weina; Li, Haigang; Zhang, Junling; Shen, Jianbo; Brown, Hamish; Wang, Enli (2022): Contrasting patterns of accumulation, partitioning, and remobilization of biomass and phosphorus in a maize cultivar. In *The Crop Journal* 10 (1), pp. 254–261. DOI: 10.1016/j.cj.2021.02.014.

Zhao, Yusheng; Gowda, Manje; Liu, Wenxin; Würschum, Tobias; Maurer, Hans P.; Longin, Friedrich H. et al. (2012): Accuracy of genomic selection in European maize elite breeding populations. In *Theor Appl Genet* 124 (4), pp. 769–776. DOI: 10.1007/s00122-011-1745-y.

Zhou, Yaping; Sommer, Mauritz Leonard; Hochholdinger, Frank (2021): Cold response and tolerance in cereal roots. In *J Exp Bot* 72 (21), pp. 7474–7481. DOI: 10.1093/jxb/erab334.

Zhu, Xintian; Leiser, Willmar L.; Hahn, Volker; Würschum, Tobias (2021): Phenomic selection is competitive with genomic selection for breeding of complex traits. In *Plant phenome j.* 4 (1), e20027. DOI: 10.1002/ppj2.20027.

Please note that the following material is only available in digital form, retrievable via indicated journal links and additionally stored on the CD enclosed with this dissertation:

Publication 1: Optimizing the P balance: How do modern maize hybrids react to different starter fertilizers?

TABLE S7 Hybrids_RawDat.
Available at: https://doi.org/10.1371/journal.pone.0250496.s007 (XLSX)

Publication 2: Unraveling the potential of phenomic selection within and among diverse breeding material of 1 maize (*Zea mays* L.)

TABLE S1 Phenotypic raw data and Best Linear Unbiased Estimates of each genotype (BLUEs).
Available at figshare: https://doi.org/10.25387/g3.16692409. 9

TABLE S2 Genotypic raw data of the 50K SNP array.
Available at figshare: 11 https://doi.org/10.25387/g3.16692409. 12

TABLE S3 NIRS raw data between wavelengths of 1 250-2 400 nm, based on seed samples and based on 14 biomass samples of seedlings.
Available at figshare: https://doi.org/10.25387/g3.16692409.

Publication 3: How can we breed for phosphate efficiency in maize (*Zea mays* L.)?

TABLE S2 Raw Data Phosphate Efficiency Measures
(not yet publicly available)

List of all publications:

Peer-reviewed

Zhou, Zhipeng; Li, Guoliang; Tan, Siyu; Li, Dongdong; Weiß, Thea Mi; Wang, Xiangfeng et al. (2020): A QTL atlas for grain yield and its component traits in maize (*Zea mays*). In: *Plant Breed* 139 (3), S. 562–574. DOI: 10.1111/pbr.12809.

Li, Dongdong; Chen, Zhe; Wang, Meng; Leiser, Willmar L.; Weiß, Thea Mi; Zhao, Zheng et al. (2021a): Dissecting the phenotypic response of maize to low phosphorus soils by field screening of a large diversity panel. In: *Euphytica* 217 (1). DOI: 10.1007/s10681-020-02727-2.

Lux, Peter E.; Schneider, Jeanine; Müller, Franziska; Wiedmaier-Czerny, Nina; Vetter, Walter; Weiß, Thea M. et al. (2021): Location and Variety but Not Phosphate Starter Fertilization Influence the Profiles of Fatty Acids, Carotenoids, and Tocochromanols in Kernels of Modern Corn (*Zea mays* L.) Hybrids Cultivated in Germany. In: *Journal of agricultural and food chemistry* 69 (9), S. 2845–2854. DOI: 10.1021/acs.jafc.0c07571.

Weiß, Thea Mi; Leiser, Willmar L.; Reineke, Alice-J; Li, Dongdong; Liu, Wenxin; Hahn, Volker; Würschum, Tobias (2021): Optimizing the P balance: How do modern maize hybrids react to different starter fertilizers? In: *PloS one* 16 (4), e0250496. DOI: 10.1371/journal.pone.0250496.

Li, Dongdong; Wang, Haoying; Wang, Meng; Li, Guoliang; Chen, Zhe; Leiser, Willmar L. et al. (2021b): Genetic Dissection of Phosphorus Use Efficiency in a Maize Association Population under Two P Levels in the Field. In: *International Journal of Molecular Sciences* 22 (17), S. 9311. DOI: 10.3390/ijms22179311.

Würschum, Tobias; Weiß, Thea M.; Renner, Juliane; Friedrich Utz, H.; Gierl, Alfons; Jonczyk, Rafal et al. (2022): High-resolution association mapping with libraries of immortalized lines from ancestral landraces. In: *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik* 135 (1), S. 243–256. DOI: 10.1007/s00122-021-03963-3.

Weiß, Thea Mi; Zhu, Xintian; Leiser, Willmar L.; Li, Dongdong; Liu, Wenxin; Schipprack, Wolfgang et al. (2022): Unraveling the potential of phenomic selection within and among diverse breeding material of maize (*Zea mays* L.). In: *G3 (Bethesda, Md.)* 12 (3). DOI: 10.1093/g3journal/jkab445.

Oehme, Leon Hinrich; Reineke, Alice-J; Weiß, Thea Mi; Würschum, Tobias; He, Xiongkui; Müller, Joachim (2022): Remote sensing of maize plant height at different growth stages using UAV-based digital surface models (DSM). In: *Agronomy* 12 (958). Online verfügbar unter https://doi.org/10.3390/agronomy12040958.

Weiß, Thea Mi; Li, Dongdong; Roller, Sandra; Liu, Wenxin; Hahn, Volker; Leiser, Willmar L.; Würschum, Tobias: How can we breed for phosphate efficiency in maize (*Zea mays* L.)? In: *[manuscript submitted]*.

Articles

Thea Mi Weiß, Willmar Leiser, Tobias Würschum (2021): Welche Rolle spielt die Sortenwahl? [What role does the choice of variety play?] DLG Saatgutmagazin Winter 2021, pp. 10-12

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Curriculum vitae

Thea Mi Weiß

7th of June 1991 in Tübingen

Education

11/2018 – 04/2022:	University of Hohenheim, Institute of Plant Breeding, Germany Doctoral candidate in the Sino-German International Research Training Group "Adaptation of maize-based food-feed-energy systems to limited phosphate resources"
10/2015 – 11/2017:	University of Hohenheim, Germany MSc 'Crop Sciences - Plant Breeding and Seed Sciences'
10/2016 – 09/2017:	KWS Ferdinand von Lochow Scholarship
10/2015 – 09/2017:	Scholarship holder of the Edmund-Rehwinkel-Stiftung, Landwirtschaftliche Rentenbank
09/2011 – 06/2014:	ETH Zurich, Switzerland BSc ETH Agr 'Agricultural Sciences'
09/2001 – 06/2010:	Uhlandgymnasium Tübingen

Practical Experience

06/2018 - 10/2018:	Corteva Agriscience Temporary position assistant maize breeder Carcarès-Sainte-Croix, France
04/2017 – 08/2017:	KWS UK MSc thesis and internship, Thriplow, United Kingdom
10/2015 – 12/2016:	State Plant Breeding Institute at the University of Hohenheim Scientific assistant in the department of Rye and Fusarium, Stuttgart, Germany
05/2015 – 08/2015:	Kuebler crop farm Internship, London, Canada
02/2015 – 03/2015:	Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Internship in the department of Breeding Research – Pathogen Stress Genomics, Gatersleben, Germany
10/2014 – 12/2014:	Africa RISING Internship, Research in Sustainable Intensification for the Next Generation, Bunda College, Lilongwe, Malawi
06/2013 – 07/2013:	Affolter no-till-farm with alpine farming Internship, Bern, Switzerland
04/2011 – 06/2011:	European Parliament

	Internship with Elisabeth Jeggle, MEP, member of the Committee on Agriculture and Rural Development, Brussels, Belgium

Social Engagement

02/2019 – present:	Voluntary work at retirement home Cusanus-Haus
09/2019 – 09/2021	University of Hohenheim Member of the Senate
WS 2013/SS 2014:	ETH Zurich Board member for university policy of VIAL, student association of agronomists and food scientists
11/2011 – 03/2014:	Wohnheim Zürichberg Work with disabled people, 'Verein Züricher Eingliederung'
07/2008 – 06/2010:	youth-life-line Peer consultant for young people at risk of suicide, 'Arbeitskreis Leben e.V.'

22nd of April 2022, Hohenheim

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Declaration in lieu of an oath on independent work

according to Sec. 18(3) sentence 5 of the University of Hohenheim's Doctoral Regulations for the Faculties of Agricultural Sciences, Natural Sciences, and Business, Economics and Social Sciences

1. The dissertation submitted on the topic

Assessing the genetic variation of phosphate efficiency in European maize (*Zea mays* L.)

is work done independently by me.

2. I only used the sources and aids listed and did not make use of any impermissible assistance from third parties. In particular, I marked all content taken word-for-word or paraphrased from other works.

3. I did not use the assistance of a commercial doctoral placement or advising agency.

4. I am aware of the importance of the declaration in lieu of oath and the criminal consequences of false or incomplete declarations in lieu of oath.

I confirm that the declaration above is correct. I declare in lieu of oath that I have declared only the truth to the best of my knowledge and have not omitted anything.

Hohenheim, 22nd of April 2022

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