Phosphorous Efficiency and Tolerance Traits for Selection of Sorghum for Performance in Phosphorous-Limited Environments

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ABSTRACT

Sorghum (*Sorghum bicolor* (L.) Moench) is widely cultivated in West Africa (WA) on soils with low phosphorus (P) availability. Large genetic variation for grain yield (GY) under low-P conditions was observed among WA sorghum genotypes, but information is lacking on the usefulness of P-tolerance ratios (relative performance in –P [no P fertilizer] vs. +P [with P fertilizer] conditions) and measures of P-acquisition and internal P-use efficiency as selection criteria for enhancing GY under low-P conditions. We evaluated 70 WA sorghum genotypes for GY performance under –P and +P conditions for 5 yr in two locations in Mali and assessed P acquisition (e.g., P content in biomass) and P-use efficiency (e.g., grain produced per unit P uptake) traits under –P and +P conditions in one site in 2010. Significant genetic variation existed for all P-tolerance ratios across multiple sites. Photoperiod-sensitive landrace genotypes showed significantly better P tolerance and less delay of heading under P-limited conditions compared with photoperiod-insensitive varieties. Genotypic correlations of P-tolerance ratios to GY under –P were moderate. Phosphorous acquisition and P-use efficiency traits independent of harvest index were of similar importance for GY under –P conditions in statistically independent trials. However grain-P and stover-P concentrations from one –P trial showed only weak correlations with GYs in statistically independent trials. Highest predicted gains for –P GY were obtained by theoretical index selection based on –P GY combined with P-use efficiency traits (e.g., low-grain P concentration). Such index selection is expected to achieve both increased sorghum productivity and P sustainability in the P-limited WA production systems.

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Abbreviations: +P, with P fertilization; −P, without P fertilization; BLUP, best linear unbiased prediction; BMY, total biomass yield; GCV, genetic coefficient of variation; GY, grain yield; GYR, grain-yield ratio; h^2, broad sense heritability; HDR, heading-date ratio; ICP-OES, inductive-coupled plasma optical-emission spectrometer; PBM, P content of total biomass; PCG, P concentration in grain; PCS, P concentration in stover; PG, grain P content; PHI, P harvest index; PHR, plant-height ratio; PS, P content of stover; PUTIL-BM, P utilization for biomass production; PUTIL-G-Hi, P utilization for grain production adjusted for harvest index; PUTIL-G, P utilization for grain production; PUTIL-S, P utilization for stover production; SVR, seedling-vigor ratio; SY, stover yield; WA, West Africa.

S OIL P deficiency is a major constraint to sorghum productivity in WA (Buerkert et al., 2001). Low plant-available P field conditions have significantly reduced seedling vigor, plant height, and GY and show a delay in flowering (Rossiter, 1978; Nord and Lynch, 2008; Leiser et al., 2012b). Delayed flowering and reduced plant height may expose WA sorghum to greater risks of end-of-season water deficit and grazing by transhumant cattle. Sorghum in WA is extensively cultivated under low-input conditions due

to the preponderance of highly weathered soils with low plant available P (Doumbia et al., 1993), farmers' limited access to fertilizer (van der Velde et al., 2013), and sorghum's capacity to produce yields with no or limited fertilizer inputs (Leiser et al., 2012b, 2014). The adaptation of sorghum to low-P conditions is thus crucial for food security and livelihoods in the Savannah zone of WA.

Plants evolved two basic adaptation strategies for soils with low plant-available P levels: higher P-acquisition efficiency from soils and improved internal physiological P-use efficiency (Vance et al., 2003; Richardson et al., 2011). A high P-acquisition efficiency is generally indicated by a high P content of the biomass, whereas internal P-use efficiency is defined as the biomass unit produced per P unit. Soil-based screening studies generally confound both of these adaptation strategies (Rose and Wissuwa, 2012). The performance of a genotype grown under low-P conditions relative to its performance under high-P conditions can be considered as its P tolerance (Chaubey et al., 1994). Phosphorous tolerance for seedling vigor scores could indicate a genotype's resilience or sensitivity to P-limited conditions at establishment, with early root growth and relative amounts of seed-born P reserves being possible determinants (Veneklaas et al., 2012). Phosphorous tolerance for plant height and heading date may reflect the cumulative effects of P limitation on a genotype's cumulative growth up to the flowering period due to reduced rates of stem elongation, reduced leaf initiation, or leaf appearance rates. Phosphorous tolerance for GY may represent the integration of mechanisms for adaptation to limited P availability throughout the vegetative and reproductive growth periods. Additionally, the extent of photoperiod sensitivity of WA sorghums was shown to be related to the level of adaptation to low-P conditions (Leiser et al., 2014).

While P-acquisition efficiency is considered a major adaptation mechanism, especially in soils with a high P-retention potential as is common in WA (Kochian, 2012), a higher internal P-use efficiency could help limit soil-P mining, particularly in low-input farming systems (Rose and Wissuwa, 2012). Both adaptation strategies show a large genotypic variation in WA sorghum and several other crops (Rossiter, 1978; Fageria et al., 1988; Atlin and Frey, 1989; Wissuwa and Ae, 2001; Manske et al., 2001; Turk et al., 2003; Chen et al., 2008; Cichy et al., 2008; Parentoni et al., 2010; Leiser et al., 2014). However, the extent of genetic variation and genotype-by-environment interaction for P-tolerance ratios is not currently known for sorghum.

Several studies in different crops, including WA sorghum, investigated the importance of P acquisition and internal P-use efficiency for GY production and concluded that P acquisition is much more important for GY production under low-P conditions (Jones et al., 1989; Manske et al., 2001; Araújo and Teixeira, 2003; Ozturk et al., 2005; Cichy et al., 2008; Parentoni et al., 2010; Leiser et al., 2014). However, the determinations of P acquisition, P-use efficiency, and GY in the same environments, as was the case in the above-mentioned studies, risks autocorrelations that may mask the true genotypic relationships. Validation of these relationships using GYs from an independent set of environments is thus crucial for guiding applied breeding for low-P conditions. An opportunity to conduct such a validation exists with the multiyear, multilocation characterization of diverse WA sorghums under low- and high-P conditions (Leiser et al., 2012b) along with the detailed assessment of P acquisition and P-use efficiency of these same genotypes (Leiser et al., 2014). Additionally, there is no known examination of the potential utility of P-tolerance ratios or of the potential value of an index selection for P acquisition or P-use efficiency traits together with low-P GY, for discriminating among WA sorghums for their genetic value for GY under low-P conditions.

This study is intended to guide breeding for enhanced GY under P-limited conditions by (i) characterizing the diversity (genetic variation and heritabilities) for P-tolerance ratios among a diverse set of WA sorghum genotypes and (ii) to assess the potential value of P-tolerance, P acquisition, and internal P-use efficiency traits as selection criteria per se or as components of an index selection for enhancing genetic values for low-P GY by (a) examining correlations with GYs from a series of independent low-P environments, (b) estimating predicted GY responses to alternative selection criteria, and (c) retrospectively assessing the performance levels for P acquisition and internal P-use efficiency traits of genotypes selected for superior GY across multiple low-P environments.

MATERIALS AND METHODS
Field Trials
The 70 sorghum varieties used in this study represent the diversity of cultivars and breeding lines adapted to the Sudanian zone of Mali. Approximately half of the entries were landrace varieties belonging to the Guinea-race, being tall (stem–internode length ≥20 cm) with the majority having intermediate- to high-photoperiod sensitivity (Supplementary Table S1). The remaining entries were bred from biparental Caudatum and Guinea–Caudatum race crossing and backcrossing, or from a Guinea-race, random-mating population. These bred varieties represent a continuum from Guinea- to Caudatum-race phenotypes for grain- and glume-characteristics, with diversity for plant height, and intermediate to nonsensitive photoperiod sensitivities (Supplementary Table S1). Further details on the genetic material was described by Leiser et al. (2012b, 2014).

Field trials were conducted in Mali, West Africa, at the Samanko (12°31′ N, 8°4′ W) station of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and at the Kolombada (12°40′ N, 7°0′ W) research station of the Institut d'Economie Rurale for 5 yr from 2006 to 2010. All trials were

rain–fed, with two separate adjacent trials sown on the same day at each location and year: one with P fertilization, denoted +P, and one without, denoted −P, as described in Leiser et al. (2012b).

Seedling vigor at approximately 25 d after sowing was observed using a visual score ranging from 1 (least) to 9 (most vigorous), whereby corresponding −P and +P trials were scored on the same day using the same scale. The date of heading when 50% plants had emerged panicles was noted in Julian days. Plant height, defined as the distance from ground to the panicle tip, was measured on three plants per plot and averaged. Plots were harvested at maturity, with panicles dried at ambient temperatures with daily maximum of 40°C for a minimum of 2 wk before weighing panicles and threshing grain. The genotypic performance of the P-tolerance (−P/+P) ratios was assessed by computing for each genotype a seedling–vigor ratio (SVR), heading-date ratio (HDR), plant-height ratio (PHR) and grain–yield ratio (GYR) whereby adjusted genotypic mean values, or best linear unbiased predictions (BLUPs), of the −P trial were divided by the corresponding +P trial adjusted genotypic means for the respective traits. Genotypes showing higher SVR, PHR, and GYR and lower HDR ratios are expected to be better adapted to low-P.

One trial, Samanko 2007 +P, failed and two other trials, Kolombada 2008 −P and Samanko 2008 +P, were excluded from the analysis due to environmental factors resulting in small differences for GY, plant height, and date of heading between the corresponding −P and +P trials (Leiser et al., 2012b). No field trial was conducted at Kolombada in 2010. Therefore, P-tolerance ratios could not be calculated for 2008, nor for Samanko 2007 and Kolombada 2010, leading to six −P and +P trial pairs for P-tolerance trait analysis and to 15 single trials (eight −P and seven +P) for GY analysis. Although plant vigor scores were recorded in all trials, only data from 2009 and 2010 were used since only in these 2 yr a consistent scale was used for scoring across −P and +P trials, hence only three −P and +P trial pairs could be used for final plant vigor analysis.

Several measures for P acquisition (here referred to as P uptake) and internal P-use (here referred to as P-use) efficiency of each genotype were calculated (Table 1) for the −P and +P trials in Samanko 2010. Stover and grain samples of mature plants were harvested from the whole plot, air dried until no weight changes were observed, and a sample of 500 g was ground to fit a mesh sieve of 0.5 mm. Due to difficulty of measuring the very large stover biomass under the +P conditions, only two of the four replications were analyzed, whereas all four replications under −P were evaluated. Phosphorous concentrations of stover and grain samples were analyzed using an inductive coupled plasma optical emission spectrometer (ICP-OES) as described in VDLUFA (2011). All grain samples were also analyzed for their Al content using ICP-OES to determine any possible soil contamination, since Al is generally not taken up into grain tissue by sorghum.

Data Analysis

Each trait in each trial was separately analyzed with a mixed model restricted maximum likelihood (REML) analysis considering the genotype factor as fixed and replication and incomplete block factors as random. If this incomplete-block analysis did not yield repeatability values of ≥0.75, several

Table 1. Phosphorus concentration, uptake, and use-efficiency traits of sorghum evaluated in the Samanko 2010 −P and +P trials.

Trait	Description	Calculation	Unit
GY	Grain yield		t ha⁻¹
SY	Stover yield		t ha⁻¹
BMY	Total biomass yield	GY + SY	t ha⁻¹
PCG	P concentration in grain		mg P g⁻¹
PCS	P concentration in stover		mg P g⁻¹
PG	P content of grain	PCG × GY	kg P ha⁻¹
PS	P content of stover	PCS × SY	kg P ha⁻¹
PBM	P content of total biomass	PG + PS	kg P ha⁻¹
PHI	P harvest index	PG/PBM	%
PUTIL-G	P utilization for grain production	GY/PBM	kg g⁻¹ P
PUTIL-G-Hi	PUTIL-G adjusted for harvest index	see Materials and Methods section	kg g⁻¹ P
PUTIL-S	P utilization for stover production	SY/PBM	kg g⁻¹ P
PUTIL-BM	P utilization for biomass production	BMY/PBM	kg g⁻¹ P

spatial models were fitted as described in a companion study (Leiser et al., 2012a). The optimum model was identified based on Akaike's information criterion, and predicted values and standard errors were computed for each genotype, taking the factor genotype as fixed. For the analysis of grain P concentration (PCG) and grain P content (PG) in −P, we included grain Al content as covariable as fixed regression factor to account for possible contamination of the seeds with soil. This was not necessary in +P since no significant Al effect was detected. Phosphorous utilization for grain production with adjustment for harvest index (PUTIL-G-Hi) was calculated using harvest index as a fixed regression factor in a mixed model to estimate P utilization for grain production (PUTIL-G) independent of harvest index (Leiser et al., 2014).

To dissect the genotype-by-environment interaction variance component from the error variance component in a two-stage, multi-environment analysis of the P-tolerance ratio traits, the variance of each genotype ratio in each environment (location–year combination) was calculated based on the delta method as stated by Johnson et al. (1993). Since both trials (−P and +P) were separate trials in adjacent fields, we can assume independence of both trials conditionally on the environment, which is a prerequisite for using this method:

$$\text{var}(A/B) = \left(\frac{A}{B}\right)^2 \times \left(\frac{\text{var}(A)}{A^2} + \frac{\text{var}(B)}{B^2}\right),$$

where A and B are the adjusted means in −P and +P conditions, respectively, and var(A) and var(B) are the variances of the corresponding adjusted mean per genotype and environment in −P and +P conditions, respectively.

A two-stage, multi-environment combined analysis was conducted to estimate the sources of variance across multiple environments. A combined weighted mixed-model REML analysis was applied. The model can be stated as follows:

Table 2. Grand mean, genotypic minimum, maximum, broad sense heritability (h^2), genetic coefficient of variation (GCV), and variance components (σ^2 for genotype [G], environment [E], and genotype-by-environment interactions [GE] and multiplied by 1000 for better readability ± standard error [SE]) for seedling-vigor ratio (SVR), heading-date ratio (HDR), plant-height ratio (PHR), and grain-yield ratio (GYR) between –P and +P conditions across three to six –P and +P environmental pairs (P_E).

–P/+P Ratios	P_E	Mean	Min	Max	h^2	GCV	$\sigma^2_G \pm$ SE	$\sigma^2_{GE} \pm$ SE	$\sigma^2_E \pm$ SE
SVR	3	0.36	0.32	0.39	0.36	6.80	0.60 ± 0.23	0.00 ± 0.00	1.00 ± 1.06
HDR	6	1.07	1.01	1.17	0.95	3.24	1.20 ± 0.24	0.60 ± 0.00	1.70 ± 1.00
PHR	6	0.77	0.72	0.81	0.48	2.90	0.50 ± 0.20	1.00 ± 0. 24	3.90 ± 2.48
GYR	6	0.51	0.32	0.74	0.73	14.93	5.80 ± 1.59	4.60 ± 1.20	13.10 ± 8.49

$$Y_{ij} = \mu + G_i + GE_{ij} + E_j + e_{ij} ,$$

where Y_{ij} is the trait ratio of the ith genotype in the jth environment, μ is the grand mean, G_i is the effect of the ith genotype, E_j the effect of the jth environment, GE_{ij} the effect of the interaction between G_i and E_j, and e_{ij} is the error of each Y_{ij} considered by the reciprocal variance as described in Leiser et al. (2012b). The weighted mixed-model analysis allowed us to model heterogeneous error variances and to separate the error variance from the genotype-by-environment variance component (Piepho, 1999). The variance of e_{ij} was modeled by var(A/B) as explained above. Broad sense heritabilities were estimated based on Cullis et al. (2006).

The potential usefulness of various traits to serve as selection criteria for enhancing GY under low-P conditions was assessed, first, by computing Pearson correlations of genotypic means for each trait assessed (e.g., P uptake, P-use efficiency) in the Samanko 2010 −P and +P environments, respectively, with GYs in each of seven independent −P environments, and with GY BLUPs estimated over all seven independent −P environments. Second, we simulated selection of the 10% best genotypes for their yield, P uptake and P-use efficiency performance under −P conditions in Samanko 2010 and for their P-tolerance ratios observed in Samanko 2010. These selected 10% best genotypes were then compared with the overall performance of the entire set of genotypes for their mean GY estimates (BLUPs) across the seven independent −P trials by subtracting the grand mean GY performance from the mean performance of the selected group. Selection in Samanko 2010 −P was conducted in accordance with the observed correlations, with the 10% lowest ranking genotypes selected for traits showing a negative relationship to the −P GY BLUPs and the 10% highest ranked genotypes selected for traits with positive correlations. Further, selection was also based on an index that combined −P GY together with a second trait according to the following index:

$$\text{Index}_i = GY_{-Pi} + \text{Trait}_{-Pi}$$

where Index$_i$ is the index value for the ith genotype, GY_{-Pi} is the standardized value of GY of the ith genotype, and Trait$_{-Pi}$ the standardized value of the companion trait of the ith genotype, both from the Samanko 2010 −P trial. Where the companion trait was negatively related to GY the standardized value of the companion trait was subtracted for computing the index (Index$_i$ = GY_{-Pi} − Trait$_{-Pi}$).

Table 3. Correlations of genotypic values of P-tolerance (–P/+P) ratios for seedling vigor (SVR), heading date (HDR), plant height (PHR) with grain-yield ratio (GYR) with each ratio derived from a combined analysis over all evaluated environments, and correlations of genotypic values for all four ratios with genotypic predictions of grain yield across six –P environments (–P GY$_{BLUP}$).

Trait	GYR	–P GY$_{BLUP}$[†]
SVR	0.60***	0.47***
HDR	−0.51***	−0.26*
PHR	0.53***	0.43***
GYR	−	0.74***

* Significantly different from zero in two-sided t-test at the 0.05 probability level.

*** Significantly different from zero in two-sided t-test at the 0.001 probability level.

[†] BLUP, best linear unbiased prediction.

RESULTS
Genetic Variation of Phosphorous-Tolerance Ratios

Significant genotypic variance was observed for all four P-tolerance ratios examined (Table 2). Grain–yield ratios showed more than a two-fold range and the highest genetic coefficient of variation (GCV), followed by SVR with a much lower genetic variation. The mean heading date was delayed by 5.9 d in −P vs. +P conditions across all analyzed trial pairs, with genotype BLUPs ranging from 1.5 to 13.2 d delay. Whereas broad sense heritabilities across environments were high for HDR and GYR, they were much lower for SVR and PHR. Although GYR showed a rather large significant genotype-by-environment interaction variance component, the genotypic variance component was larger than that for genotype-by-environment interaction (G/GE = 1:0.79), as was the case for all traits examined except PHR. Best linear unbiased predictions across all environments were therefore used in further analyses (Table 3, Fig. 1). Landrace varieties showed a significantly higher P tolerance for GY (GYR = 0.54) compared with that of research bred lines (GYR = 0.49). Landrace genotypes also exhibited significantly less delay of heading under −P conditions (HDR μ = 1.06, with an absolute mean delay of 4.4 d) compared with the researcher-bred genotypes (HDR μ = 1.09, and 7.4 d mean delay). The extent of heading delay differed considerably depending on the degree of photoperiod
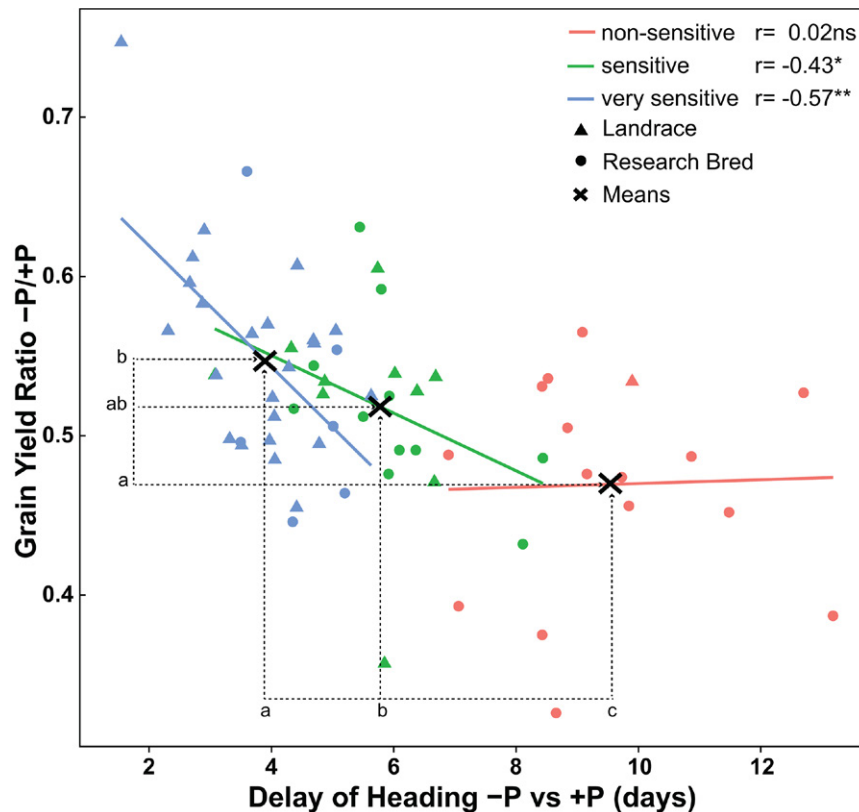
Figure 1. Relationships between delay of heading (–P vs. +P) and grain-yield ratio (GYR; –P/+P) over six –P +P environmental pairs of 66 West African sorghum (*Sorghum bicolor* (L.) Moench) genotypes classified by degree of photoperiod sensitivity as detailed in Supplemental Table S1 and Leiser et al. (2012b) (different colors) and selection history (landrace vs. research bred; different symbols). Photoperiod sensitivity class means, indicated with ×, show significant differences at $p < 0.05$ (Tukey-test) when labeled with different letters. The genotype groups, landrace and research-bred, are significantly different ($p < 0.05$) from each other for delay in heading and GYR in a two-sided *t*-test.

sensitivity (Fig. 1), with nonsensitive genotypes showing most delay and highly sensitive genotypes the least delay. Average P tolerance for seedling vigor of landrace varieties (SVR = 0.37) was nearly identical, although differing significantly, from that of research bred lines (SVR = 0.36).

Relationship of Phosphorous-Tolerance Ratios and Grain Yield

Heading delay ratio was significantly negatively related to GYR and –P GY BLUPs across the whole set of genotypes (Table 3), but as previously described, this differed according to the degree of photoperiod sensitivity (Fig. 1). The highly photoperiod sensitive genotypes, although having the least –P heading delay and yield reduction, showed a strong negative relationship between HDR and GYR. In contrast, the nonsensitive genotypes had more heading delay and greater relative yield reductions but showed no relationship between the two. Heading delays within each photoperiod sensitivity class did not show any significant correlations to GY BLUPs across –P as well as across +P environments (data not shown). Seedling-vigor ratios and PHR had positive significant correlations to both GYR and GY BLUPs across the –P environments. Seedling-vigor ratios had the strongest relationship to

GYR among the three measures of P tolerance. However, GYR had the highest correlation with –P GY BLUPs if GYRs were derived from many of the same environments (partial autocorrelation; Table 3) but showed only a correlation of $r = 0.31$ ($p < 0.05$) to –P GY BLUPs if the GYRs were derived from Samanko 2010, thus being independent from the other environments.

Relationship of P-Uptake and P-Use Efficiency Traits with Grain Yield in P-Limited Conditions

Whereas P-uptake measures (P content of total biomass [PBM], P content of grain [PG], and P content of stover [PS]) showed strong positive correlations with GY in independent –P environments, many of the P-use efficiency and P-concentration traits showed weaker to no relationships (Table 4). However, P utilization for biomass production (PUTIL-BM) and PUTIL-G-Hi had correlations to –P GY of similar strength as the P-uptake traits. Although P concentration in stover (PCS) under –P conditions was unrelated to –P GY BLUPs, PCS under +P conditions showed a highly significant negative correlation. The corresponding partitioning of P between grain and vegetative tissue (P harvest index [PHI]) showed the reverse pattern,

Table 4. Correlations of genotypic means for measures of yield, P uptake, use efficiency, concentration, and partitioning assessed in a single –P or +P environment with grain yields in seven independent –P environments (minimum, maximum, mean), and with grain yield (GY) best linear unbiased predictions (BLUPs) estimated over all seven independent –P environments.

Trait[†]	–P				+P			
	Min	Max	Mean	–P GY$_{BLUP}$	Min	Max	Mean	–P GY$_{BLUP}$
BMY	0.45	0.73	0.58	0.75***	0.15	0.54	0.41	0.50***
GY	0.35	0.62	0.52	0.68***	0.32	0.57	0.45	0.59***
SY	0.32	0.68	0.52	0.65***	0.03	0.46	0.33	0.40**
PBM	0.31	0.62	0.49	0.62***	0.04	0.45	0.28	0.34**
PG	0.33	0.60	0.46	0.59***	0.36	0.47	0.44	0.56***
PS	0.20	0.55	0.41	0.52***	−0.15	0.34	0.13	0.14ns[‡]
PUTIL-BM	0.26	0.52	0.39	0.51***	0.13	0.32	0.22	0.26*
PUTIL-G-Hi	0.15	0.47	0.34	0.46***	0.20	0.52	0.36	0.48***
PUTIL-S	0.19	0.50	0.35	0.44***	0.00	0.25	0.15	0.18ns
PUTIL-G	−0.19	0.29	0.01	0.04ns	−0.03	0.50	0.19	0.27*
PCS	−0.11	0.06	−0.01	−0.02ns	−0.38	−0.12	−0.29	−0.37**
PCG	−0.42	0.07	−0.13	−0.20(*)	−0.41	0.06	−0.17	−0.24(*)
PHI	−0.43	−0.14	−0.31	−0.39**	−0.07	0.35	0.11	0.16ns

(*) Significantly different from zero in two-sided *t*-test at the 0.10 probability level.

* Significantly different from zero in two-sided *t*-test at the 0.05 probability level.

** Significantly different from zero in two-sided *t*-test at the 0.01 probability level.

*** Significantly different from zero in two-sided *t*-test at the 0.001 probability level.

[†] See Table 1 for trait abbreviation descriptions.

[‡] ns, not significantly different from zero.

with PHI under −P significantly (negatively) correlated to −P GY BLUPs whereas PHI from +P was unrelated.

The traits showing the strongest correlations with −P GY BLUPs were measures of total biomass yield (BMY) and GY evaluated under −P conditions in Samanko 2010. These yield traits, even when observed under +P conditions, showed large significant correlations (Table 4). Nevertheless, the −P traits showed higher correlations and consistency (lower average variance of the correlations, data not shown) across the independent −P environments compared with the traits observed under +P.

Selection exercises identifying the 10% highest ranking genotypes for each of the traits examined in Samanko 2010 resulted in mostly superior GY estimates, based on mean GY BLUPs of the selected genotypes relative to the grand mean over the seven independent −P environments (Table 5). Selection based on high GY in Samanko −P and low HDR (derived from Samanko 2010) gave the highest GY superiority with 22.7 and 20.5 g m^{-2} yield advantages over the grand mean of 88.5 g m^{-2}, respectively (Table 5). Use of an index composed of GY and a companion trait increased GY superiority relative to the companion trait per se. In fact, indices including BMY, PCG, PUTIL-G-Hi, PUTIL-S, PUTIL-BM, and HDR as companion traits produced GY superiorities that were numerically, but nonsignificantly, higher than GY per se selection. Furthermore, the indices including PCG and PUTIL-BM showed smaller standard deviations among the 10% best genotypes compared with GY per se, indicating a higher consistency for GY selection.

Table 5. Absolute grain yield superiority relative to the grand mean (± σ in g m^{-2}) over seven independent –P environments of the 10% best performing genotypes selected on the basis of Samanko 2010–P traits per se (yield, P-uptake, P-use efficiency) and Samanko 2010 P-tolerance (–P/+P) ratios and on a selection index composed of standardized values of Samanko 2010 –P grain yield (GY) and companion trait.

	Trait	Trait per se	Index (GY ± trait)
Yield[†]	GY	22.7 ± 13.5	–
	SY	6.9 ± 20.7	19.9 ± 18.6
	BMY	13.9 ± 20.3	26.1 ± 13.5
P-uptake[†]	PCG	4.9 ± 22.2	25.1 ± 11.4
	PCS	−2.4 ± 21.4	20.6 ± 18.0
	PG	12.0 ± 21.3	18.3 ± 16.4
	PS	2.3 ± 15.4	19.0 ± 19.2
	PBM	14.3 ± 20.2	22.7 ± 13.5
P-use[†]	PHI	0.3 ± 14.5	22.7 ± 12.3
	PUTIL-G	1.0 ± 27.1	16.3 ± 19.4
	PUTIL-G-Hi	8.6 ± 19.8	24.3 ± 13.5
	PUTIL-S	10.5 ± 18.6	25.3 ± 16.6
	PUTIL-BM	14.9 ± 18.3	27.1 ± 11.1
P-tolerance[‡]	SVR	3.1 ± 14.8	11.8 ± 19.6
	HDR	20.5 ± 17.4	23.5 ± 14.7
	PHR	1.2 ± 21.7	18.4 ± 19.3
	GYR	14.9 ± 22.6	14.4 ± 22.1

[†] See Table 1 for trait abbreviation descriptions.

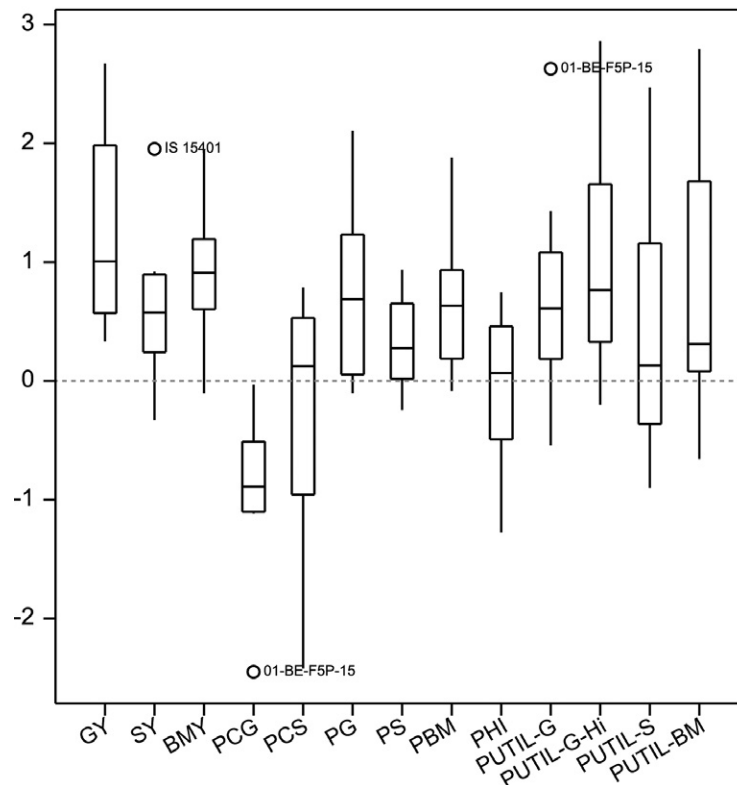[‡] GYR, grain-yield ratio; HDR, heading-date ratio; PHR, plant-height ratio; SVR, seedling-vigor ratio.

Figure 2. Standardized values ($\mu = 0$, $\sigma = 1$) for measures of yield, P concentration, P-uptake and P-use efficiency of mature sorghum (*Sorghum bicolor* (L.) Moench) plants in −P conditions in Samanko 2010 (for trait abbreviations see Table 1) of the 10 highest yielding genotypes identified across eight −P environments. The reference line at 0 corresponds to the mean of all 66 genotypes for these traits. The box spans the interquartile range of the values in the trait, so that the middle 50% of the data lie within the box with a line indicating the median. The whiskers extend at a distance of 1.5 times the interquartile range beyond the quartiles or the maximum value, if that is smaller. Outliers are indicated with labels showing the genotype names.

Retrospective of P-Uptake and P-Use Efficiency of Highest Yielding Genotypes

The 10 genotypes with highest GYs over multiple −P environments (Leiser et al., 2012b) generally exhibited both superior P uptake (PG, PS, PBM) and P-use efficiency, especially PUTIL-G and PUTIL-G-Hi, and inferior PCG values (Fig. 2). Nevertheless, there was variation among these genotypes for P uptake, PUTIL-G, and PCG with ranges from near zero to over one standard deviation. No trends for PCS, PHI, and PUTIL-S were observable among the highest yielding genotypes, with wide trait ranges straddling the mean (0.0).

The one genotype that exhibited the strongest specific adaptation to low-P conditions (Leiser et al., 2012b) among the 10 top ranked genotypes for −P yields, the Guinea landrace IS15401, had only intermediate P-uptake rates in the −P environment relative to the other nine genotypes. The genotype that exhibited highest yields across all low-P environments, the research-bred variety NafalenP6, showed the second highest P-uptake values among the 10 best low-P-adapted genotypes. Both IS15401 and NafalenP6 had superior PHI, PUTIL-G, and PUTIL-G-Hi values and lower PCG and PCS values relative to the mean of the 10 best −P yielding genotypes. Nevertheless,

IS15401 had by far the highest stover yield and the highest PUTIL-BM among the best genotypes. In contrast, the second-ranked genotype for GY across all low-P environments, the researcher-bred variety Tiandougou, showed very high P-uptake rates, but low P-use efficiencies with values below the overall average for PUTIL-BM and just above average for PHI and PUTIL-G-Hi.

DISCUSSION

Diversity of Phosphorous-Tolerance Ratios and Their Usefulness to Select for Yield Performance under Low-Phosphorous Conditions

Deficiency of P is known to cause reduced and delayed growth in sorghum (Sahrawat et al., 1995; Leiser et al., 2012b), as was observed in this study across six pairs of −P and +P environments. Early plant development (e.g., seedling vigor) was most affected by P deficiency, indicated by the smallest ratio of −P relative to +P conditions. Early plant development is highly dependent on P nutrition (e.g., soil, seed), which may directly contribute to final yield production (Grant et al., 2001; Valluru et al., 2010). Although we did not measure the seed P

concentration of the actual sown seeds, we suggest that the higher seedling vigor of landraces and highly photoperiod sensitive genotypes may be due to their generally higher grain P concentrations (Leiser et al., 2014), which is critical for early plant establishment under low-P conditions (White and Veneklaas, 2012). Nevertheless, further research is necessary to clarify this relationship, especially since simulation studies showed that P concentration in the grain might be reduced to minimize soil-P mining, without having any negative impact on early plant establishment (Rose and Wissuwa, 2012). Although SVR showed a highly significant correlation to GY, its use as indirect selection measure in early stages is not advisable due to its lower broad sense heritability (h^2) and GCV (Table 2) and only moderate correlation to absolute GY production in independent −P conditions.

Grain-yield ratio, although showing the highest genetic variance, acceptably high h^2, and proportionally small genotype-by-environment interaction, appears to be less promising as a selection criterion than GY per se under −P based on our simulated selection results (Table 5). Using GYR as the only selection trait will increase specific adaptation to −P conditions but can also lead to a lower general yield potential (Blum, 2005), since GYR and GY in −P showed correlations ranging only from $r = 0.46$ ($p < 0.001$) to $r = 0.69$ ($p < 0.001$) with GY in single −P environments (data not shown) and $r = 0.74$ ($p < 0.001$) GY −P BLUPs across all six environments (Table 3). The relationship was even lower ($r = 0.31$; $p < 0.05$) if GYRs were calculated in a single environment (Samanko 2010) and correlated to GY −P BLUPs estimated across statistically independent −P environments. Therefore, use of GYR for selection is less promising than −P GY per se, which had a higher h^2 and genetic variance across the six −P trials ($h^2 = 0.91$; GCV = 22.1) than GYR and would avoid the additional cost of testing under +P as well as −P conditions to estimate GYR. Thus, the present study confirmed that direct selection for GY in −P is advisable to optimize selection response in −P target environments as noted by Leiser et al. (2012b). Nevertheless, genotype evaluation under both −P and +P conditions could be useful to determine yield potential across the range of production systems in WA and evaluate the heading delay and plant height reduction under −P conditions. Heading-date ratio and PHR, although not necessarily of direct use for genotypic −P grain selection (lower GCV, weak correlation), may be useful to reduce risks of poor grain filling with an early ending of seasonal rains (Dingkuhn et al., 2006) and transhumant livestock damage of shorter height plants.

Photoperiod Sensitivity as an Adaptation Mechanism for Low-Phosphorous Soils and Variable Climates

Since flowering and heading date show high heritabilities, the delay of heading in −P relative to +P would seem to offer a simple measure for a genotypes' capacity to grow under P-limited conditions and possibly its adaptation to variable (earlier) end of rains. Although −P heading delay was fairly strongly and significantly correlated to GYR for more photoperiod sensitive germplasm (Fig. 1), the lack of correlation of −P heading delay with −P GY in the non-photoperiod sensitivity group cautions against its direct use as a selection criterion. There was confounding of maturity and −P heading delay in our panel, with less sensitive genotypes being early maturing and showing more delay, whereas photoperiod-sensitive genotypes were later maturing with less delay. The heading delay of the less-sensitive germplasm showed no negative consequences in our test environments, possibly due to these genotypes being too early for this zone under +P conditions with a June sowing. The later maturing, more photoperiod-sensitive genotypes, appear to have more specific adaptation to −P conditions, but this is not likely due to small −P heading delays but, in part, may reflect less yield response to +P. The low-P tolerance of this germplasm group nevertheless is of interest for production under current P-scarcity conditions and possible future increase in climate variability (Dingkuhn et al., 2006; Haussmann et al., 2012).

The better P tolerance (Fig. 1) and higher P-acquisition efficiency (Leiser et al., 2014) of photoperiod-sensitive genotypes might be due to their bilinear rates of aboveground growth (with leaf appearance, organ initiation, and stem elongation slowing after reaching 25 plastochrons) (Clerget et al., 2008), whereas rooting depth continues at a constant rate (Clerget, personal communication, 2006), suggesting a higher P-uptake potential per unit of aboveground growth at later stages of growth. Differences between early and late P uptake of a plant may have different consequences for final GY production under low-P conditions (Jones et al., 1992). Hence the question of how P uptake and its balance with aboveground growth change over time and germplasm type could be especially pertinent for WA sorghums noting the mix of photoperiod-sensitive and insensitive varieties and the diversity and plasticity of aboveground growth patterns. Although our study focuses solely on final P status, major differences were observed between nonsensitive and highly photoperiod-sensitive germplasm for P uptake and P-use efficiency traits (Leiser et al., 2014), suggesting that different growth patterns could have an impact on early and late P uptake and thus on adaptation to P-limited environments.

Use of Uptake- and Use-Efficiency Traits to Select for Yield Performance under Low Plant-Available Phosphorous Conditions

We examined the utility of P uptake and P-use efficiency traits for breeding for −P GY from two angles: (i) correlations with −P GY in a statistically independent set of −P environments (Table 4) and GY superiorities of genotypes identified by simulating selection on these traits (Table 5) and (ii) expression of these traits by genotypes with the highest −P GY (Fig. 2). Whereas the highest yielding −P-adapted genotypes showed both high P uptake (PG, PS, PBM) and high P-use efficiency (with high PUTIL-G, PUTIL-G-Hi, and low PCG), the correlations showed P-uptake traits to be most related to −P GY. Both P uptake and P-use efficiency are crucial for crop adaptation to low-P soils (Sattelmacher et al., 1994; Wang et al., 2010; Rose and Wissuwa, 2012). Studies in different crops, including one study in sorghum (Leiser et al., 2014), investigating the importance of P uptake and P-use efficiency for GY production conclude that P-uptake is much more important for GY production under low-P conditions (Jones et al., 1989; Manske et al., 2001; Araújo and Teixeira, 2003; Ozturk et al., 2005; Cichy et al., 2008; Parentoni et al., 2010). These studies however are prone to autocorrelations since the P parameters and GY were from the same environments. The correlations in this study, based on GY from independent environments, nevertheless also showed more importance of P-uptake traits for final GY production.

The absence of an observed relationship between PUTIL-G and −P GY across our full panel may suggest that the elevated PUTIL-G values of our best −P-adapted genotypes may reflect sampling and not necessarily causative relationship. Furthermore, PUTIL-G in such a diverse panel is strongly confounded with harvest index (Rose et al., 2011; Rose and Wissuwa, 2012; Leiser et al., 2014), which was shown to be negatively correlated to GY production under −P conditions ($r = -0.27$; $p < 0.05$). Looking at P-use measures independent of harvest index (PUTIL-BM, PUTIL-G-Hi, and PCG), we observed correlations to −P GY of similar magnitude as for P-uptake traits, suggesting that internal P-use efficiency is of importance for final GY production.

Phosphorous uptake and P-use efficiency traits showed higher heritabilities than GY in Samanko 2010 −P (Leiser et al., 2014) and thus could be considered as possible traits for indirect selection for higher −P GYs. However, the rather weak correlations to GY in independent −P environments limit the effectiveness of these traits per se for indirectly selecting for −P GY, as indicated by their slightly lower GY superiorities (Table 5). Yet combining these traits with GY in a selection index may be useful for selecting P-efficient genotypes with high yields. For example, including P-use measures independent of harvest index (including PCG) with GY in a selection index resulted in higher absolute GY estimates (Table 5), thus suggesting that internal P-use efficiency can contribute to final GY production under P-limited conditions. Such index selection is advisable, especially when targeting both increased −P GY as well as minimized soil P mining, which is occurring in most sub-Saharan African regions (Stoorvogel et al., 1993). Nevertheless, estimating these P-use efficiency traits costs time and money and might not lead to significant improvements compared with a simple selection solely based on GY under −P conditions. One P-use efficiency measure, which is of broader importance and rather easily measured, is P concentration in the grain. Hence this trait could be a viable complementary trait for −P GY selection.

While higher P concentration in the grain can be of importance for early plant development, especially on low-P soils (White and Veneklaas, 2012), this needs to be balanced with advantages of lower grain-P concentrations for minimizing soil-P mining and reducing antinutritional phytic acid content in the grain, which inhibits Zn and Fe uptake by humans (Hurrell et al., 2003). As PCG showed only a weak correlation ($r = -0.2$) to −P GY, selection for lower PCG may be conducted with no yield penalty. Such selection could be highly pertinent since more than 70% of total P is found in the grain of mature sorghum plants grown under low-P conditions (Leiser et al., 2014) and would be removed from the farm nutrient cycle when sold in urban markets. Combined selection for low-PCG and high-GY using a selection index resulted in increased −P GY estimates (Table 5) and is thus expected to simultaneous improve yield and reduce PCG.

Although P uptake appears generally more important for enhancing WA sorghum GY under −P, as shown for other crops (Vance et al., 2003), the examination of the P uptake and P-use efficiency trait levels of genotypes with the highest −P GYs indicated that they had diverse strategies. Some of these superior genotypes had high P uptake and low P-use efficiency, while others had relatively low P uptake and high P-use efficiency (Fig. 2; data not shown).

The strong correlation of BMY with −P GY (Table 4), numerically higher than GY per se, may indicate that total biomass, as the cumulative expression of above-ground crop growth, integrates multiple components and thus may be of value for predicting adaptation to −P environments. Since BMY in −P showed the highest and most consistent (low standard deviation) correlation to −P GY across many −P environments, it could be used as selection criteria, especially if only few field trials can be conducted. The lack of significant correlation between BMY and PCG but positive correlations with heading date ($r = 0.6$, $p < 0.001$) indicate that selection for BMY could result in indirect selection for lateness but not for PCG. Thus, use of GY per se as a direct selection criterion or in an index combined with P-use efficiency traits (e.g., PUTIL-G-Hi, PCG, PUTIL-BM) is likely more useful, especially if later flowering is undesirable.

Finally, P uptake or P-use efficiency traits from −P conditions are more useful than from +P environments for indirectly selecting for −P GY due to generally higher correlations to −P GY (Table 4) and similar high heritability values in −P and +P conditions (Leiser et al., 2014). Additionally, the lower standard deviations of the −P GY correlations (Table 4; data not shown) for −P traits compared with +P traits suggest that selection under −P conditions is less prone to false selection due to large environmental variation. Thus, the trait relationships observed in this study over independent environments support the conclusion of Leiser et al. (2012b) that direct selection under −P conditions is more effective for improving −P GY than indirect selection under +P conditions. Further, the correlation of −P GY (Samanko 2010) with GY BLUPs across +P and −P environments was $r = 0.66$ ($p < 0.001$), in comparison with $r = 0.61$ ($p < 0.001$) of +P GY (Samanko 2010), respectively (data not shown). These results indicate the need to emphasize testing under P-limited conditions by West African sorghum breeding programs in general and especially when targeting the needs of the majority of farmers for whom sorghum production under limited fertilization is vital for food security and income.

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