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Phosphorus uptake and utilization efficiency in West African pearl millet inbred lines

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ABSTRACT

Pearl millet [*Pennisetum glaucum* (L.) R. Br] production on the acid sandy Sahelian soils in West Africa (WA) is severely limited by low plant-available phosphorus (P) in addition to erratic rainfall. We sought to examine the genetic variability for P uptake and P utilization efficiency in 180 WA pearl millet inbred lines or subsets thereof under low (LP) and high P (HP) conditions in one field and two pot experiments, determine the relationships among the measured traits and grain yield under field conditions at three other independent WA sites, and identify potential secondary selection traits for improving grain yield under LP. We observed genetic variation for P uptake and utilization in both seedling and mature plants. P utilization efficiency increased under LP conditions. Total P uptake was more important for grain production than P utilization under LP field conditions ($r = 0.57^{***}$ vs $r = 0.30^{***}$). The estimated response to indirect selection was positive for most of the measured morphological and P-efficiency parameters. We conclude that both seedling and mature plant traits are potentially useful as secondary traits in selection of pearl millet for low-P adaptation. These results should be validated using heterozygous pearl millet genetic materials. Ultimately, pearl millet breeding activities for low P tolerance in WA should be integrated with other system-oriented research such as nutrient cycling, intercropping or rotations with legumes, better crop-tree-livestock integration, and modest applications of locally available rock phosphate.

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1. Introduction

Low phosphorus (P) soils are a major constraint to crop production in West Africa (WA). Pearl millet, a staple crop in WA, is predominantly cultivated by small-holder farmers on low-input sandy soils, thus adaptation to those edaphic conditions is crucial for food security in this region (Shen et al., 2011). Fertilizer application rates are very low in WA with levels mostly below $5\,kg\,P\,ha^{-1}$ (Obersteiner et al., 2013). Furthermore, most of the WA soils are highly weathered low pH soils with a high P retention level (Kochian, 2012), thus fixing most (70–90%) of the applied P as plant unavailable phosphate (Holford, 1997), hence most small-holder farmers' fields within WA have plant available P levels of the soil below the critical level of $7\,mg\,P\,kg^{-1}$ soil (Manu et al., 1991; Doumbia et al., 2003). Enhancing phosphorus efficiency (PE) in pearl millet would offer an affordable option for improving yields in low-input farming systems (Ismail et al., 2007; Wissuwa et al., 2009; Rose et al., 2011) and would serve especially small-holder farmers in WA. PE can be defined as the ability to acquire nutrients from the soil and utilize them for biomass and/or grain yield production (Gerloff, 1977; Manske et al., 2000). It is therefore constituted by P uptake efficiency (ability to acquire P from the soil) and P utilization efficiency (the amount of biomass and/or grain yield produced per unit P in the plant), with the contribution of these two aspects of PE depending on crop species and environmental conditions (Manske et al., 2000; Rose et al., 2011; Wang et al., 2010).

Being an immobile element in the soil, P concentration in the soil solution is usually much less than $0.3\,mg\,P\,L^{-1}$ and often as low as $0.001\,mg\,P\,L^{-1}$ whereas concentrations in plant tissues could be as high as $300\,mg\,P\,kg^{-1}$ (Bieleski, 1976; Manske et al., 2000). This makes the uptake of P from the soil very slow (Fitter and Hay, 2002; Hammond et al., 2004). P deficiency in the plant initiates a series of transcriptional, biochemical and physiological responses which either enhance the plant's ability to acquire P from the soil or improve the efficiency with which plants utilize P internally (Hammond and White, 2008). Such responses include the development of lateral roots and root hairs, as well as more dramatic root structures such as proteoid and dauciform roots, the secretion from roots of phosphatases and organic acids, and the induction of high-affinity and some low-affinity inorganic phosphate (Pi) transporters as well as establishing symbiotic associations with mycorrhizal fungi that aid P acquisition (Burleigh et al., 2002; Lambers et al., 2006; Ai et al., 2009; Fang et al., 2009; Yang and Finnegan, 2010).

Low-P tolerance in WA pearl millet has been studied mainly based on morpho-physiological attributes (Bationo et al., 1993; Buerkert et al., 2001; Brück et al., 2003; Faye et al., 2006; Beggi et al., unpublished) but most of these studies were only based on a few genotypes. Genetic variation has been reported for rooting parameters as well as fertilizer response in WA pearl millet (Manga and Saxena, 1988; Brück et al., 2003; Faye et al., 2006). Furthermore, Beggi et al. (unpublished) have shown genotypic differences for PE under pot trial conditions in WA pearl millet landrace varieties. The usefulness of morphological or physiological traits in a breeding programme depends on their genetic correlation with grain yield, extent of genetic variation, heritability, genotype-by-environment interactions and costs of assessment (Mir et al., 2012). According to Reynolds and Trethowan (2007), the use of such traits therefore requires proper definition in terms of stage of crop development at which they are relevant, the specific attributes of the target environment for which they are adaptive, and their potential contribution to yield. P availability has been shown to be critical in the early developmental stages (Barry and Miller, 1989; Rebafka et al., 1993; Hajabbasi and Schumacher, 1994; Plenet et al., 2000; Valluru et al., 2010) and therefore, P deficiency in early stages is a direct

constraint for crop production, particularly under agricultural conditions where intensive soil fertilization is not affordable (Lynch, 1995; Calderon-Vazquez et al., 2008). PE can therefore be evaluated both at early and adult plant stages. Early growth stage evaluation would allow for the use of managed environment phenotyping which is thought to reduce environmental variability associated with field experiments thereby reducing the error variance and improving heritability and precision (Blum, 2011; Rebetzke et al., 2013). However, it is also accepted that in spite of the notorious heterogeneity in the fields, controlled experiments, especially under pot trial conditions, are usually far removed from the situation the plant would find itself in the field. This makes the results from controlled environments difficult to extrapolate to field conditions (Araus and Cairns, 2014) because the phenome is dynamic, conditional, with complex responses and a wide range of endogenous and exogenous signals integrated over the evolutionary and developmental life history of an individual (Houle et al., 2010; Cobb et al., 2013).

To date, no study has specifically focused on WA inbred lines as breeding materials and the genetic variation among them for several PE measures under pot and field conditions. Furthermore, information is lacking on the relationship between genotype performance at an early growth stage in pots and genotype performance of mature plants in the field. The objectives of the current study therefore were:

(i) to examine the genetic variation for low P tolerance related morphological traits, P uptake and P utilization efficiency in WA pearl millet inbred lines at an early growth stage in pot experiments and at maturity under field conditions under low and high P conditions;

(ii) to determine the relationships among the measured traits and grain yield from independent environments; and

(iii) to determine which traits show potential for use in indirect selection for grain yield under low-P.

2. Materials and methods

2.1. Genetic materials

One hundred and eighty inbred lines (IBLs) of pearl millet [*Pennisetum glaucum* (L.) R. Br. syn *Cenchrus americanus* (L.) Morrone] were developed from a collection of landraces from West and Central Africa to represent a large part of the diversity of pearl millet in this region, which is also the centre of origin for pearl millet. The list and details of the 160 inbred lines included in the field trials is shown in Supplementary Table 1. The inbred lines were at the fourth selfing generation (S_4) in the rainy season (RS) 2011 and the fifth selfing generation (S_5) in RS 2012. In the RS 2011, 180 inbred lines were evaluated in a pot experiment at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Sahelian Centre, Sadore, Niger. For the field experiments at a total of four locations, only 160 inbred lines among them could be included (due to seed shortage of some lines for field experiments owing to inbreeding depression). The same seed was used for both pot and field trials. In RS 2012, a subset of 52 contrasting genotypes were selected based on grain yield data from the four locations, and a second pot experiment was set up. No corresponding field trial was carried out in RS 2012.

2.2. Field trials

In the rainy season 2011, 160 inbred lines were evaluated at Sadore, Niger (17 36′ 28.04″N; 8 4′ 53.99″W) together with two checks in separate trials planted side by side under high

Table 1
Descriptions, abbreviations and units of traits measured in the field and in pot experiments on pearl millet inbred lines.

	Description	Abbreviation	Calculation	Unit
Field	Seedling vigor	SV		1–5 scale
	Height at 4 weeks	HT4		cm
	Height at 6 weeks	HT6		cm
	Time to flowering	FLO		days
	Stover weight	SWT		$g\,m^{-2}$
	Panicle weight	PWT		$g\,m^{-2}$
	Grain yield at Sadore	SGY		$g\,m^{-2}$
	P concentration in stover	PCS		$mg\,g^{-1}$
	P concentration in grain	PCG		$mg\,g^{-1}$
	Total above ground biomass	BM	SWT + PWT	$g\,m^{-2}$
	Grain harvest index	HI	$(GY/BM) \times 100$	%
	Total P in grain	PG	$PCG \times GY$	$mg\,m^{-2}$
	Total P in stover	PS	$PCS \times SWT$	$mg\,m^{-2}$
	Total P in biomass	PBM	PG + PS	$mg\,m^{-2}$
	P concentration in biomass	PCBM	PBM/BM	$mg\,g^{-1}$
	P harvest index	PHI	$(PG/PBM) \times 100$	%
	P harvest index to grain harvest index	PHI:HI	PHI/HI	
	P utilization efficiency	PUTE	GY/PBM	$g\,mg^{-1}\,P$
Pot	Seedling vigor	SV		1–5 scale
	Height	HT		cm
	Number of leaves	NL		
	Number of tillers	NT		
	Stem diameter	SD		cm
	Root dry biomass	RDB		g
	Shoot dry biomass	SDB		g
	Root to shoot ratio	RS	RDB/SDB	
	P concentration in shoot	PCS		$mg\,g^{-1}$
	Total P in shoots	PS	$PCS \times SDB$	mg
	P utilization efficiency	PUTE	SDB/PS	$g\,mg^{-1}\,P$
	Relative shoot growth −P/+P	RSG	SDB_{LP}/SDB_{HP}	

phosphorus (HP; with P fertilization) and low phosphorus (LP; without P fertilization) conditions in a 18×9 α-lattice with three replications in each treatment. Soil samples were taken before sowing from the top 20 cm depth and analyzed in the soil laboratory of ICRISAT at Sadore, Niger. The soil analysis data revealed an average of pH-H_2O = 4.6; total nitrogen = 175 mg N kg^{-1} soil; organic carbon = 0.21%; Bray-1 P = 3.3 mg P kg^{-1} soil and exchangeable potassium ions (K$^+$) = 0.12 cmol$^+$ kg^{-1} soil for the HP trial and pH-H_2O = 4.6; total N = 184 mg N kg^{-1} soil; organic carbon = 0.20%; Bray-1 P = 3.4 mg P kg^{-1} soil and K$^+$ = 0.12 cmol$^+$ kg^{-1} soil for the LP trial. The HP treatment received 100 kg ha^{-1} diammonium phosphate (DAP) corresponding to 20 kg ha^{-1} P and 18 kg ha^{-1} N at sowing, whereas the LP treatment received a basal application of 39 kg ha^{-1} urea corresponding to 18 kg ha^{-1} N. Both treatments were then supplied with two topdressings (30 and 45 days after sowing) of 35 kg ha^{-1} urea corresponding to 16 kg ha^{-1} N. The trials were fully raised as rainfed. In each of the trials, a single row of 7 hills per plot with 0.8 m intra- and 0.75 m inter-row spacing was used so as to result in a final plot area of 3.6m^2. For grain yield evaluation only, the same trial was also planted in three other locations: Gampela, Burkina Faso (12 25′ 51″N; 1 22′ 18″W); Bambey, Senegal (14 42′ 2.66″N, 16 27′ 32.8″W); and Koporo, Mali (14 3′ 49.9″N; 3 4′ 31″W) under the same soil fertilization treatments (Gemenet et al., 2014). Data collected or computed include (Table 1): seedling vigor at three weeks after sowing on a scale of 1–5 (SV; 1 = very poor, 5 = excellent), plant height measured from the base of the plant to the tip of the longest leaf from three representative plants per plot at four (HT4, cm) and six (HT6, cm) weeks after sowing, time to flowering (FLO, days) measured as the number of days from sowing until 50% stigma emergence on main panicles per plot, stover weight (SWT, gm^{-2}) measured by air-drying stover per plot to constant weight and then weighing, panicle weight (PWT, gm^{-2}) by measuring the weight of all panicles per plot and then grain yield (GY, gm^{-2}). In addition, P concentration in stover (PCS, mg g^{-1}) and grain (PCG mg g^{-1}) were analysed using an inductive coupled plasma emission spectrometer (ICP-OES) according

to VDLUFA (2011) and other parameters were calculated based on these data (Table 1).

2.3. Pot trials

In 2011, 180 inbred lines were evaluated under HP and LP in a pot trial at Sadore, Niger starting from 1st of September, 2011. The experiment was set up in a randomized complete block design (RCBD) with 3 replications per P-level. A second pot experiment was carried out in 2012 from October 1st with a subset of 52 genotypes and two checks. The 52 genotypes were selected based on grain yield data under LP (2011) from the four locations (Sadore in Niger, Gampela in Burkina Faso, Koporo in Mali and Bambey in Senegal) with 26 high yielding and 26 low yielding genotypes. The trial was also laid in RCBD with 4 replications per P-level. For both experiments, soil filled into the pots was taken from the top 20 cm soil from the same LP field used for the field trial at Sadore. The soil composition in 2011 was as given above for field trials. In 2012, only pH-H_2O and Bray 1 P (mg P kg^{-1}) were analysed and had average values of 4.36 and 3.16, respectively. To set up the experiments, the soil was dried, sieved and mixed for homogeneity. The pots with a diameter of 0.28 m and a height of 0.25 m were filled with 0.5 kg gravel to facilitate drainage, followed by 10 kg of soil each. For the HP treatment, 50 mg P kg^{-1} soil and 45 mg N kg^{-1} soil in the form of DAP was applied per pot at sowing. To balance the N in the LP treatment, 45 mg N kg^{-1} soil in the form of urea was applied per pot. Nine seeds were sown in 3 holes per pot, with the fertilizer hole in the center. At 7 days after sowing, pots were thinned to two plants per pot. The plants were raised for six weeks exclusively under irrigation to field capacity every other day. At two, four and six weeks after sowing the following traits were measured as the average of the two plants in the pot: seedling vigor (SV, 1–5 scale: 1 = very poor, 5 = excellent), height (HT, cm), stem diameter (SD, cm), number of leaves (NL), number of tillers (NT). At the end of six weeks, all above ground biomass was harvested and the roots washed off from the soil on nets to allow recovery of most fine roots.

Table 2

Predicted means (μ) of 160 pearl millet inbred lines, repeatabilities (w^2), coefficients of genetic variation (CV_g) and the estimated level of error (aVD) for traits measured in the field (Sadore 2011) under low phosphorus (LP) and high phosphorus (HP) conditions and the genotypic variance component (G), genotype by P-level variance component ($G \times T$) and genotypic correlations (r_g) across HP and LP.

Trait[a]	LP				HP				Combined		
	μ	w^2	CV_g	aVD	μ	w^2	CV_g	aVD	G [b]	$G \times T$[b]	r_g
SV	2.7	0.65	14.1	11.8	2.9	0.72	14.4	10.7	0.19***	0.018*	0.98
HT4	46.3	0.62	14.1	12.3	50.1	0.67	13.7	11.1	42.9***	4.23*	0.98
HT6	102.9	0.74	20.4	14.6	112.7	0.68	16.2	12.8	360.2***	30.3*	0.97
FLO	65.5	0.89	6.9	3.2	61.3	0.88	6.5	3.2	17.9***	0.26 ns	0.98
SWT	147.2	0.61	36.7	32.0	158.9	0.67	38.8	31.4	3297***	701*	0.94
BM	180.3	0.60	19.8	17.5	191.0	0.75	9.8	6.8	6956***	1099*	0.97
HI	28.8	0.40	13.7	14.8	29.2	0.61	19.3	17.0	25.85***	2.92*	0.93
SGY	53.8	0.59	22.0	26.0	58.8	0.84	29.3	17.9	3.87***	0.94*	0.96
PCS	0.61	0.71	20.7	15.6	0.72	0.67	17.1	13.8	0.05***	0.002 ns	0.89
PCG	2.26	0.56	9.4	7.7	2.48	0.72	10.1	7.5	0.07***	0.011*	0.83
PS	70.8	0.64	13.4	11.1	109.6	0.73	29.5	21.3	4185***	455*	0.97
PG	101.6	0.53	10.2	9.8	188.4	0.79	29.9	19.4	3357***	722*	0.96
PBM	211.7	0.59	21.7	19.2	258.6	0.76	25.5	17.6	13795***	1342*	0.95
PCBM	1.1	0.35	4.0	4.4	1.28	0.51	12.0	11.8	0.021***	0.002 ns	0.91
PHI	58.1	0.56	15.4	14.0	56.2	0.70	18.3	14.0	36.59***	5.39*	0.87
PHI:HI	2.1	0.53	14.6	14.0	2.0	0.64	18.8	15.8	0.12***	0.0022*	0.84
PUTE	0.26	0.63	18.4	15.8	0.23	0.72	22.0	16.3	0.0024***	0.0001*	0.93

[a] Trait abbreviations and units shown in Table 1.
[b] *, **, *** Significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

The roots and shoots were then dried in a green house to constant moisture and weighed for shoot dry biomass (SDB) and root dry biomass (RDB). Root to shoot ratio (RS) was calculated as RDB/SDB. In addition, shoot P concentration (PCS) was analysed according to VDLUFA (2011), only for the 180 genotypes evaluated in 2011, and other parameters were calculated from these data as shown in Table 1.

2.4. Statistical analyses

In single environment analysis, the repeated measures option in REML mixed models was used to analyze genotype performance using the uniform correlation/split-plot in time correlation model within time points and allowing heterogeneity over time, for all traits measured at intervals in pots. Genotypes as well as replications were considered random whereas time was considered fixed. For non repeatedly measured traits in pots as well as all traits from the field experiment, linear mixed models were used. Genotypes as well as replications (pot data) or blocks nested in replications (field data) were considered random. Across P-levels, P fertilization treatment was considered fixed whereas genotypes, genotype by P-level interactions as well as block and/or replication effects were considered random. Repeatability estimates (w^2) were calculated as $w^2 = \sigma^2_g / [\sigma^2_g + (VD/2)]$ where VD is the average variance of a difference between means of genotypes and σ^2_g is the genetic variance component (Piepho and Möhring, 2007). To allow for comparison of genetic variance components across trials with differing means, the genetic coefficients of variation were calculated as: $CV_g = (\sigma^2_g)^{1/2}/\mu$, where σ^2_g is the genetic variance component and μ the grand mean (Leiser et al., 2012). To be able to compare the extent of error across trials of differing mean yields, the standardized average variance of a difference (aVD) was calculated as: $aVD = (VD)^{1/2}/\mu$ where VD is the average variance of a difference between means of genotypes, and μ is the trial mean (Leiser et al., 2012). Genetic correlations (r_g) across P-levels were calculated as $r_{g(HP, LP)} = r_{p(HP\,LP)}/(w^2_{HP} \times w^2_{LP})^{1/2}$, where $r_{p(HP, LP)}$ is the phenotypic correlation of the traits measured under HP and LP, whereas w^2_{HP} and w^2_{LP} are the respective repeatability estimates (Cooper et al., 1996), for both field and pot experiments. Genotypic correlations among traits were calculated based on predicted means from field and pot trial data with grain yield predicted means from each single location [Bambey (BGY), Koporo (KGY),

Gampela (GGY), Sadore (SGY)] as well as best linear unbiased predictors (BLUPs) from combined analysis across the four locations (CGY). Two approaches were used to examine potential secondary traits for indirect selection to improve grain yield under low P. First, a trait *per se* response to selection was calculated based on the 10% best genotypes per secondary trait using BLUPs combined across the four LP locations (CGY) as SR = μ-select − μ-pop, where μ-select is the mean of the selected individuals at 10% selection intensity and μ-pop is the grand mean (population mean). The 10% best genotypes were selected based on their genotypic correlation with grain yield such that the highest ranking 10% were selected for those traits positively correlated with GY and the lowest ranking 10% for those traits negatively correlated with GY. Secondly, an index-based selection was carried out combining GY and trait as Index$_i$ = GY$_{-Si}$ + Trait$_{-Si}$ for traits positively correlated with GY and Index$_i$ = GY$_{-Si}$ − Trait$_{-Si}$ for traits negatively correlated with grain yield, where, Index$_i$ is the index value for the *i*th genotype, GY$_{-Si}$ is the standardized value of GY across four locations (CGY) of the *i*th genotype, and Trait$_{-Si}$ the standardized value of the companion trait of the *i*th genotype from the Sadore 2011 LP trial (Leiser et al. 2014b, in press). The standardized values were obtained as ($\mu_{geno} − \mu_{pop}$)/std dev, where μ_{geno} is the genotypic mean, μ_{pop} is the population grand mean and std dev is the standard deviation.

3. Results

3.1. Genetic variation of morphological traits and P-parameters under field conditions

The tested pearl millet inbred lines varied significantly for all traits measured under field conditions at Sadore in 2011 ($p < 0.001$) (Table 2). Means of traits were generally reduced under LP except for P harvest index (PHI), P harvest index to grain harvest index ratio (PHI:HI) and P utilization efficiency (PUTE) which were higher under LP than HP. Flowering was delayed by about four days under LP. P uptake (PBM) was higher under HP than LP. Repeatability estimates were generally reduced under LP, mainly due to increased error variance relative to genetic variance. The repeatabilities ranged from 0.35 to 0.85 under LP and from 0.51 to 0.88 under HP (Table 2). P concentration in total biomass (PCBM) was the least heritable trait under both HP and LP. Across P-levels, genotypes were highly different for all traits ($p < 0.001$). The P-level

Table 3
Genotypic correlations between traits measured in the field at Sadore in 2011 and grain yield (minimum, maximum and mean) from the four single locations (Sadore, Gampela, Koporo and Bambey), and combined grain yield across the locations (CGY) under low phosphorus conditions (LP) and high phosphorus conditions (HP) and the correlation between the grain yield from single environments and the combined grain yield across the four environments (CGY).

Trait[a]	LP				HP			
	Single location			Combined	Single location			Combined
	Min	Max	Mean	CGY[b]	Min	Max	Mean	CGY[b]
SV	0.46	0.74	0.58	0.65***	0.44	0.72	0.56	0.68***
HT4	0.46	0.70	0.55	0.61***	0.39	0.63	0.49	0.60***
HT6	0.47	0.71	0.56	0.63***	0.44	0.72	0.58	0.71***
FLO	−0.28	−0.47	−0.40	−0.44***	−0.29	−0.51	−0.43	−0.53***
SWT	0.41	0.75	0.49	0.52***	0.51	0.81	0.60	0.73***
HI	0.19	0.44	0.32	0.37***	0.21	0.52	0.37	0.45***
PCG	−0.36	−0.47	−0.43	−0.50***	−0.26	−0.40	−0.34	−0.42***
PBM	0.42	0.47	0.56	0.57***	0.50	0.86	0.61	0.74***
PHI	0.02	0.17	0.11	0.12 ns	0.06	0.23	0.18	0.21+
PHI-HI	−0.22	−0.39	−0.28	−0.32***	−0.20	−0.47	−0.33	−0.40***
PUTE	0.12	0.38	0.26	0.30***	0.18	0.34	0.28	0.34***
CGY	0.72	0.87	0.79	0.77***	0.77	0.85	0.82	−

[a] Trait abbreviations and units are shown in Table 1; ns = non significant.
[b] +, *, **, *** Significant at $p < 0.1$, $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

treatment was significant for all traits ($p < 0.05$). We observed significant genotype-by-P-level interaction for most traits (p < 0.05). There was a strong association between traits under HP and LP with genetic correlations across P-levels ranging from 0.83 to 0.98 (Table 2).

3.1.1. Relationships among morphological traits and P-parameters measured under field conditions with grain yield under LP

Most morphological traits and P-parameters were significantly correlated to grain yield (GY) in single locations under LP and HP (Table 3) except for P harvest index (PHI). Trait relationships with GY trends did not differ for single location grain yield and grain yield combined across the four locations (CGY; Table 3). Coefficients of correlation were a bit higher for grain yield at Sadore (SGY) for both LP (Fig. 1) and HP (data not shown) and this could be a result of autocorrelation. We therefore report relationship to grain yield based on combined grain yield best linear unbiased predictors (BLUPs) across the locations (CGY). Stover weight (SWT) was highly positively correlated to CGY under both HP ($r = 0.73***$; Table 3) and LP ($r = 0.52***$; Fig. 1, Table 3). Total P uptake (PBM) was more important for grain production than P utilization (PUTE) under both LP ($r = 0.57***$ vs $r = 0.30***$; Fig. 1, Table 3) and HP ($r = 0.74***$ vs $r = 0.34***$; Table 3). Furthermore, P uptake traits were better correlated with the measured seedling vigor (SV), plant height at four weeks (HT4), plant height at six weeks (HT6) and stover weight (SWT) than P utilization under LP (Fig. 1). The P concentration in grains (PCG) was more than three times higher than the P concentration in stover (PCS) for both LP and HP ($\mu = 2.26$ against $\mu = 0.61$, respectively, under LP; Table 2). Increased P concentration in grains (PCG) was only weakly associated with reduced P harvest index (PHI; $r = −0.14+$), whereas this association was moderately high with reduced grain harvest index (HI; $r = −0.41***$), P utilization efficiency (PUTE; $r = −0.54***$) and combined grain yield (CGY; $r = −0.50***$) under LP (Fig. 1) and ($r = 0.19+$, $r = −0.46***$, $r = −0.56***$ and $r = −0.42***$ for the respective four traits) under HP (data not shown). P harvest index (PHI) ranged from 32% to 71% under LP with a mean of 58.1%. Grain harvest index (HI) was highly significantly and positively correlated to P harvest index (PHI) and P utilization efficiency (PUTE) in LP (Fig. 1) and HP (data not shown) conditions and these are partially auto-correlations. Under LP, the ratio between P harvest index and grain harvest index (PHI:HI) was moderately negatively correlated to combined grain yield (CGY; $r = −0.32***$) and positively with P concentration with grain

(PCG; $r = 0.48***$). There was no association between P utilization efficiency (PUTE) and P uptake (PBM) under LP (Fig. 1). Total P in grain (PG) and P uptake (PBM) were positively correlated, a partial auto-correlation, under LP but there was no association between total P in stover (PS) and grain harvest index (HI) (data not shown).

3.2. Genetic variation for morphological and P-parameters under pot conditions

In the pot trials conducted at Sadore in 2011/2012, pearl millet inbred lines differed significantly for all morphological traits and P-parameters under both HP and LP conditions (Table 4). There was a significant reduction in seedling vigor, (SV), height (HT), number of leaves (NL), stem diameter (SD), root dry biomass (RDB), shoot dry biomass (SDB), shoot P concentration (PCS) and total P uptake (total P in shoots; PS) under LP conditions. Root to shoot ratio (RS) and P utilization efficiency (PUTE) increased under LP as compared to HP ($\mu = 0.8$ vs $\mu = 0.6$ for RS and $\mu = 0.9$ vs $\mu = 0.2$ for PUTE, respectively). Total P in shoots (PS) was about 8-fold increased under HP compared to LP conditions (Table 4). Relative −P/+P shoot growth (RSG) showed a substantial variation among genotypes ranging from 50% to 86% with a mean of 70% in 2011 and ranging from 30% to 78% with a mean of 60% in 2012 (Table 4). Except for seedling vigor (SV), height (HT) and number of leaves (NL) in 2011, repeatability estimates were generally reduced under LP. Repeatability estimates ranged from 0.40 to 0.75 under LP and from 0.45 to 0.75 under HP (Table 4). Across P-levels, genotypes were significantly different ($p < 0.05$) and the P-level effect was significant ($p < 0.001$) for all traits. Height (HT), root dry biomass (RDB), shoot dry biomass (SDB), root to shoot ratio (RS), P concentration in shoots (PCS), total P in shoots (PS) and P utilization efficiency (PUTE) exhibited genotype by P-level interactions ($p < 0.05$) (Table 4).

3.2.1. Relationship among early growth traits measured under LP in pot experiments and traits measured under LP in field conditions.

Most of the measured traits under pot conditions such as seedling vigor (SV), height (HT), stem diameter (SD), shoot dry biomass (SDB), root dry biomass (RDB), and root to shoot ratio (RS), were significantly correlated (Fig. 2). A higher total P uptake measured by total P in shoots (PS) was associated with increased seedling vigor (SV), stem diameter (SD), shoot dry biomass (SDB), root dry biomass (RDB) and root to shoot ratio (RS) but was negatively correlated to P utilization efficiency (PUTE) (Fig. 2). Relative −P/+P shoot growth (RSG) was positively correlated with seedling vigor (SV), height (HT), root dry biomass (RDB), shoot dry
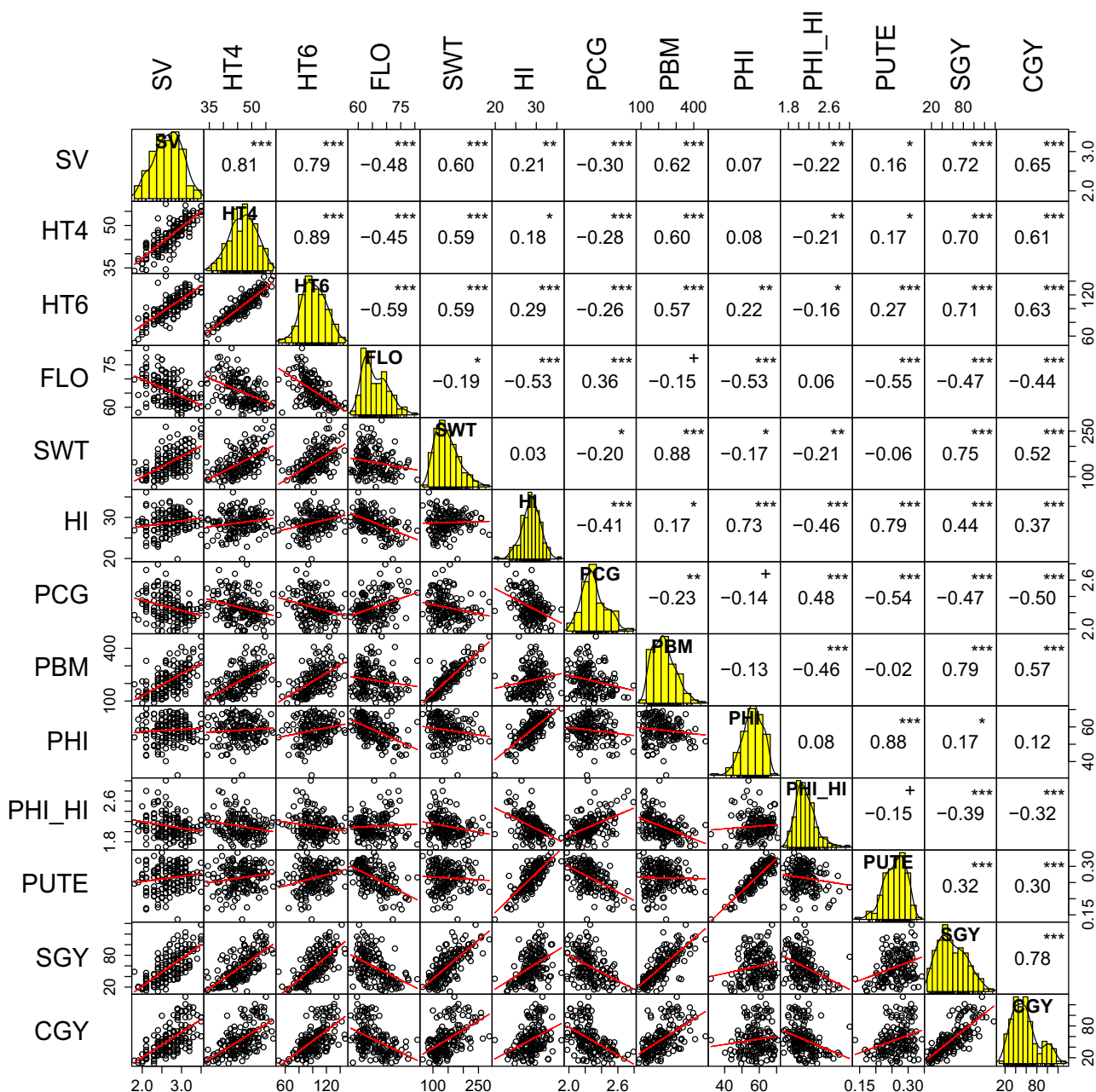
Fig. 1. Genotypic correlations among traits measured in the field under LP with grain yield at Sadore (SGY) and from three independent locations (CGY) under LP. [+], [*], [**], [***] Significant at $p < 0.1$, $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. [a] Trait abbreviations and units are shown in Table 1.

biomass (SDB), root to shoot ratio (RS), total P in shoots (PS), and P utilization efficiency (PUTE) under LP conditions, but not significantly associated with number of leaves (NL) and stem diameter (SD) (data not shown). Across P-levels, genotypic correlations were positive and significant for all traits ($p < 0.001$) (data not shown). Correlations between morphological traits and P-parameters measured in pots and grain yields from each of the four locations Bambey (BGY), Koporo (KGY), Gampela (GGY) and Sadore (SGY) followed the same trend as that of the combined grain yield across all four locations (CGY), with minimum difference between the correlation coefficients of each environment (Fig. 2). We therefore report correlation to grain yield based on combined grain yield across the four locations (CGY). Increased grain yield in the field (CGY) was associated with an increase in seedling vigor (SV), height (HT), stem diameter (SD), root dry biomass (RDB), shoot dry biomass (SDB), root to shoot ratio (RS), total P in shoots (PS),

and P utilization efficiency (PUTE), but was negatively correlated to P concentration in shoots (PCS) under pot conditions. P utilization efficiency (PUTE) under pot conditions was more correlated to combined grain yield (CGY) in field conditions under LP ($r = 0.54^{***}$; Fig. 2) as compared to HP ($r = 0.25^*$; data not shown). P concentration in shoots (PCS) under pot conditions was positively correlated with P concentration in grain (PCG; $r = 0.47^{***}$) but weakly negatively correlated to total P in stover (PS; $r = -0.28^*$), P uptake (PBM; $r = -0.31^*$) and P utilization (PUTE; $r = -0.25^+$) under field conditions (data not shown). P utilization efficiency (PUTE) under pot conditions was negatively correlated to P concentration in grain (PCG; $r = -0.39^{**}$) and weakly positively correlated with both P uptake (PBM; $r = 0.24^+$) and P utilization efficiency (PUTE; $r = 0.33^*$) under field conditions (data not shown). Total P in shoots (PS) under pot conditions and total P in stover (PS) under field conditions were not correlated.

Table 4
Predicted means (μ), repeatabilities (w^2), coefficients of genetic variation (CV_g) and estimated level of error (aVD) of traits measured on pearl millet inbred lines (180 in 2011 and 52 in 2012) in pot experiments under low phosphorus (LP) and high phosphorus (HP) conditions and the magnitude of the genotypic (G), P-level (P) and the interaction between genotypes and P-level (G × P) within two years.

Year	Trait[a]	LP				HP				Combined HP/LP		
		μ	w^2	CV_g	aVD	μ	w^2	CV_g	aVD	G[b]	P[b]	G × P[b]
2011	SV	2.0	0.75	8.4	6.7	3.1	0.58	14.3	16.8	***	***	ns
	HT	29.6	0.73	4.7	3.9	55.0	0.63	11.9	12.5	***	***	***
	NL	5.6	0.51	4.0	5.4	8.7	0.46	7.9	11.9	**	***	ns
	NT	–	–	–	–	3.3	0.58	26.8	31.4			
	SD	0.7	0.49	15.1	21.8	0.9	0.55	15.2	19.4	***	***	ns
	RDB	13.0	0.45	12.6	19.6	14.3	0.60	25.3	28.7	***	***	**
	SDB	16.5	0.58	12.4	14.6	24.0	0.56	16.0	19.0	***	***	*
	RS	0.8	0.66	3.4	3.4	0.6	0.56	11.0	13.5	**	***	*
	PCS	1.1	0.52	13.3	18.4	6.3	0.72	25.3	21.3	***	***	***
	PS	18.4	0.40	19.0	25.8	148	0.50	21.0	20.0	**	***	*
	PUTE	0.9	0.55	10.0	12.2	0.2	0.61	25.0	26.0	***	***	*
	RSG	0.7	0.65	18.4	18.7							
2012	SV	3.4	0.52	10.0	13.2	2.3	0.59	11.6	13.1	***	***	*
	HT	31.1	0.59	14.5	16.5	66.9	0.74	8.1	6.7	***	***	**
	NL	6.1	0.57	7.0	8.5	7.4	0.45	4.8	7.4	**	***	ns
	NT	0.9	0.43	43.3	69.3	3.8	0.63	20.5	21.8	**	***	ns
	SD	0.6	0.49	9.7	13.8	0.9	0.57	5.1	6.1	***	***	ns
	RDB	11.0	0.45	2.5	3.8	15.8	0.62	11.5	11.9	***	***	**
	SDB	14.1	0.49	16.9	24.2	23.0	0.75	15.2	12.2	***	***	**
	RS	0.9	0.60	29.0	32.7	0.7	0.66	19.1	18.7	***	***	*
	RSG	0.6	0.56	20.0	24.5							

[a] Trait abbreviations and units shown in Table 1.
[b] *, **, *** Significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

3.3. Efficiency of indirect selection for grain yield under LP

Most of the measured morphological and P-parameter traits under both pot and field conditions gave positive responses to selection at 10% selection intensity as traits *per se* and in an index with grain yield, except for P harvest index (PHI) and P utilization efficiency (PUTE) under field conditions which gave negative responses to selection as traits *per se* (Table 5). For morphological traits under field conditions, height at four weeks (HT4) gave the highest response to selection as trait *per se* with 34.3 gm^{-2} higher than the grand mean, followed by stover weight (SWT)

Table 5
Selection response (SR) (g m^{-2}) ± standard deviation at 10% selection intensity of the best performing pearl millet inbred lines across four locations under low phosphorus based on Sadore 2011 trait data measured under field and under pot conditions as trait *per se* and as an index between respective traits and combined grain yield across four locations (CGY).

	Trait[a]	SR (Trait *per se*)	SR (GY + trait index)
Field	CGY	54.2 ± 27.2	
	SV	30.1 ± 22.3	60.9 ± 34.1
	HT4	34.3 ± 22.6	56.4 ± 37.2
	HT6	29.1 ± 25.1	56.0 ± 42.2
	FLO	11.8 ± 29.2	37.0 ± 27.2
	SWT	33.1 ± 22.6	70.3 ± 51.2
	HI	3.8 ± 28.5	41.5 ± 23.9
	PCG	16.7 ± 27.7	41.1 ± 26.9
	PBM	29.8 ± 23.0	70.8 ± 46.0
	PHI	−10.0 ± 21.9	13.3 ± 45.0
	PUTE	−0.9 ± 31.9	32.3 ± 41.4
	PHI-HI	14.4 ± 23.8	35.3 ± 30.5
Pot	SV	21.2 ± 32.9	39.4 ± 29.9
	HT	19.1 ± 38.7	22.0 ± 31.2
	SD	17.2 ± 56.0	21.7 ± 37.2
	RDB	22.9 ± 33.4	34.4 ± 38.7
	SDB	18.9 ± 29.9	31.7 ± 35.9
	RS	24.2 ± 31.5	41.1 ± 29.0
	PCS	18.2 ± 29.2	23.0 ± 36.3
	PUTE	28.8 ± 23.7	26.6 ± 76.8
	PS	11.7 ± 40.3	79.2 ± 98.8

[a] Trait abbreviations and units are shown in Table 1.

with 33.1 gm^{-2} higher than the grand mean. For P-parameters under field conditions, P uptake (PBM) gave the highest response to selection with 29.8 gm^{-2} over the grand mean, followed by P concentration in grain (PCG) with 16.7 gm^{-2} over the grand mean as traits *per se* (Table 5). Under pot conditions P utilization efficiency (PUTE) gave the highest response to selection with 28.8 gm^{-2} above the grand mean, followed by root to shoot ratio (RS) with 24.2 gm^{-2} above the grand mean as traits *per se*. Selection based on an index combining grain yield with traits improved response to selection as compared to trait *per se* selection (Table 5). Furthermore, most morphological and P-parameters measured under field conditions gave better response to selection as compared to those measured under pot conditions (Table 5).

4. Discussion

4.1. Appropriateness of the testing conditions for assessing phosphorus efficiency

Genetic variations exist for P uptake and P utilization efficiencies in WA pearl millet inbred lines and thus this inbred line panel can be used for genetic studies and breeding for P efficiency. The significant P-level effect indicates that the two treatments somehow simulated contrasting P-fertility environments. The significant genotype-by-P-level interaction observed for most traits indicated that selection for specific adaptation to low P environments is feasible. In contrast with the results of Wissuwa and Ae (2001) and Leiser et al. (2014a), we did not observe a generalized increase in genetic variation for P uptake traits under LP relative to HP as compared to P utilization. This difference in results can be attributed to smaller differences due to less differentiation in our data set. In our sister publication (Gemenet et al., 2014), we observed low relative yield reductions between HP and LP treatments (ranging from 8.4 to 37.4% with a mean of 23.5%) across locations with high genetic correlations between the treatments (ranging from 0.87 to 0.98). Height reduction ranged from −2 to −14.7 cm. These results implied that our treatments were not sufficiently differentiated despite the supply of enough P to the HP treatment and this
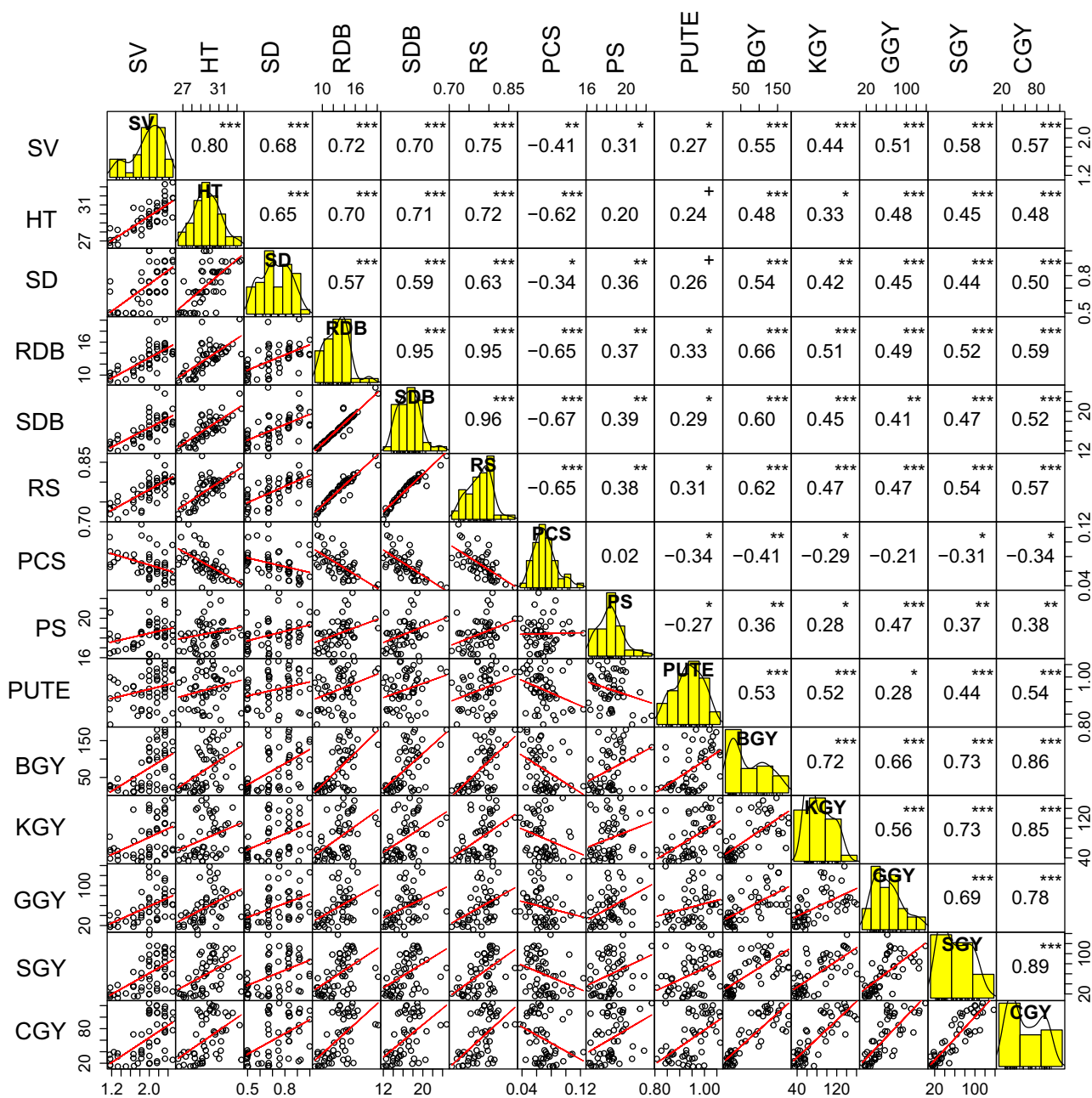
Fig. 2. Genotypic correlation among traits measured on 52 pearl millet inbred lines in pot experiments under low phosphorus with grain yield from Bambey (BGY), Koporo (KGY), Gampela (GGY), Sadore (SGY) and combined grain yield (CGY). $^+$, *, **, *** Significant at $p < 0.1$, $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. [a] Trait abbreviations and units are shown in Table 1.

indicated a masked P effect. We attributed this P effect masking to other external factors such as early-season drought experienced in 2011 that warranted late application of fertilizer treatments to avoid burning of seedlings (more than two weeks later), low soil pH that could have resulted in complexing of the fertilizer P, low initial bray-1-P values (<6 mg P kg^{-1} soil), among others. In the current study this is evidenced by the relatively lower differences in means between P-levels and a small genotype-by-P-level interaction variance relative to genotypic variance. P application is critical within the first two weeks of sowing during panicle initiation in pearl millet (Valluru et al., 2010; Vadez, 2013, personal communication) after which P application would not have much impact. This would therefore explain to a larger extend the differences in response to P treatment observed between the pot and field experiments since the pots were raised under irrigation and fertilizer

treatments could be applied at the time of sowing. In addition to this, another limitation of the study was the fact that only P and N were supplied mainly due to lack of fertilizer accessibility within the region. It is therefore highly likely that other nutrients were limiting plant growth besides P. For instance, it is shown that the sandy, siliceous, isohyperthermic Psammentic Paleustalf soils at Sadore are associated with low (<4.5) pH, high Al$^+$ saturation of the cation exchange sites, and decreased amounts of exchangeable K, Ca, and Mg. These chemical properties could result in Al or Mn toxicity or deficiencies of K, Ca, Mg, in addition to P (Scott-Wendt, 1988). We had exchangeable potassium (K) ions of 0.12 cmol+ kg^{-1} soil at Sadore in 2011 and a range of 0.09 to 0.47 cmol+ kg^{-1} soil across the sites. These facts suggest that P might not have been the main factor causing differences between our treatments. This could also offer another explanation for the observed difference in

P response between the pot experiments and the field experiments. Since pot experiments have limitations of reduced soil quantity and consequently, limited water and nutrients to the plant in addition to impeding root growth (Passioura, 2006; Poorter et al., 2012), the effects of low soil pH and possible Al toxicity and deficiencies of other nutrients could have been experienced more in pot experiments and more so in the LP pot treatment where P was also limiting.

4.2. Phosphorus uptake and utilization efficiency at maturity under field conditions

Despite the limitations on P response, we however observed a relatively higher genetic variation in P uptake (PBM) relative to P utilization (PUTE) for grain production within each P-level. A larger genetic variation for P uptake traits relative to P utilization has also been reported in wheat, maize, rice, sorghum and pearl millet (Jones et al., 1989; Wissuwa et al., 1998; Parentoni et al., 2010; Leiser et al., 2014a; Beggi et al., unpublished). P utilization (PUTE) was increased under LP conditions; hence P starvation enhances P utilization as noted by Rose et al. (2011). High GY performance was more associated with P uptake and less influenced by P utilization efficiency. This may be explained by the confounding effects of P uptake on P utilization efficiency as suggested by Rose et al. (2011). We did not observe a significant correlation between P uptake (PBM) and P utilization efficiency (PUTE), implying that these traits were independently inherited at mature plant stage and selection for either trait at this stage will not affect the other. Lack of association between P uptake and P utilization was also reported in maize by Parentoni and Souza (2008). Similar to the results of Bayuelo-Jiménez and Ochoa-Cadavid (2014) in maize landraces, we observed that differences between genotypes for P utilization efficiency were largely due to P harvest index (PHI) followed by grain harvest index (HI). However, in contrast with their findings, as well as to those by Yaseen and Malhi (2010), we observed a positive relationship between total P in grain (PG) and P uptake (PBM) and no association between total P in stover (PS) and grain harvest index (HI) indicating that in our study, more P was used in grain production rather than stover. We also found that P harvest index (PHI) as a stand alone index was unreliable for selection owing to its close correlation with grain harvest index (HI) and this implies that grain P yield is confounded by grain carbon yield (Rose et al., 2010). In fact, P harvest index (PHI) was not significantly associated with combined grain yield (CGY) but was highly associated with grain harvest index (HI). We therefore tried to separate grain P from grain carbon as suggested in Rose et al. (2010) by use of the ratio between P harvest index and grain harvest index (PHI:HI). A lower PHI:HI was associated with increased GY implying that this trait can be simultaneously selected for with GY. Another suggested ratio is that of P concentration in grain (PCG) and P concentration in total biomass (PCBM). In our study, we found this ratio having repeatability estimates of 0.53 and 0.64 under LP and HP respectively, but the P treatment effect was non-significant since the means were 2.1 and 2.0 under LP and HP, respectively. This could be due to the fact that P concentration in total biomass (PCBM) in the current data was the least heritable trait ($w^2 = 0.35$) and the applicability of this ratio should therefore be further validated. The higher P found in the grain compared to stover implies that re-translocation of P from vegetative tissues was important in grain P accumulation in our study and also signifies a large loss of P from the farming system after harvest (Rose et al., 2010). This is not desirable for Sub-Saharan African countries where fertilizer use is the least and soil P mining is taking place (Stoorvogel et al., 1993; Leiser et al., 2014a). However, it is possible to improve P internal use efficiency for example, through selecting for a lower P concentration in grain (PCG), since this trait was negatively associated with grain harvest index (HI), P utilization efficiency (PUTE) and GY. These two traits [P concentration in grain (PCG) and the ratio of P harvest index to grain harvest index (PHI:HI)] had enough genetic variation that could be exploited in breeding and they were also positively correlated. Leiser et al. (2014a) also suggested that selecting for a lower P concentration in grain (PCG) and a high P utilization efficiency for grain corrected for grain harvest index in sorghum would help reduce soil P mining within the Sahel. Despite its potentially negative impact on early growth development, (Batten and Slack, 1990; Raboy, 2009; White and Veneklaas, 2012) which needs to be further validated, low P concentration in grain (PCG) would also have a beneficial effect of reduced phytic acid in the grain thereby improving zinc–iron bio-availability that would improve micronutrient uptake especially in WA where pearl millet grain is used as a staple food (Hurrell et al., 2003; Pucher et al., 2014). The fact that Corrales et al. (2007) did not find an effect of initial seed P content on the performance of two contrasting maize genotypes under P deficiency is encouraging in this regard and the dependence of performance of young seedlings under LP on initial seed P content should be examined further in pearl millet. Despite its effects of continued soil mining, which may not be recommended for depleted soils like those in WA, P uptake efficiency as a trait cannot be neglected in plant improvement programs targeting low P environments. P uptake efficiency is necessary to improve response to P fertilization practices like the use of compost and/or manure for soil fertility maintenance. Manschadi et al. (2014) observed that genotypic selection for increasing grain yield under LP could be focused on increasing P uptake (nutrient accumulation), P harvest index (PHI; nutrient harvest index) and/or reducing P concentration in grain (PCG; % nutrient in the seed). There is therefore need to understand the trade-offs and strike for a balance between P uptake and P utilization efficiencies in a breeding program depending on soil type, soil P reserves and other soil fertility maintenance practices common in the target environment. Since most studies (Jones et al., 1989; Manske et al., 2001; Araújo and Teixeira, 2003; Ozturk et al., 2005; Cichy et al., 2008; Parentoni et al., 2010; Rose et al., 2010; Leiser et al., 2014a) focussing on P uptake and utilization efficiency have been assessed in the same field, such data are prone to autocorrelation. Leiser et al. (2014a) suggested using grain yield data from independent environments for correlations to avoid these autocorrelations. We have used grain yield data from four single locations (three of them independent) as well as combined grain yield from the four locations within WA. The significant correlation between various P-parameters such as P concentration in grain (PCG), P uptake (PBM) and P utilization efficiency (PUTE) with GY from independent environments implies the significance of the relationship between P uptake and P utilization efficiencies with GY rather than confounding effects of autocorrelations. This therefore means that there is heritable variation for these traits in the current breeding material that could be exploited in hybrid combinations. Gemenet et al. (2014) however showed only a weak relationship between inbred line performance and testcross performance of this inbred line panel implying that selection for P uptake and P utilization efficiencies directly from testcross performance rather than inbred line performance is more desirable. It remains however to be established from their study if the use of only one tester could have masked the relationship between the inbred line and testcross performance.

4.3. Phosphorus uptake and utilization efficiency at early growth stages under pot conditions

We observed genetic variation for P uptake and P utilization in the WA pearl millet inbred lines at early growth stage. Several studies have reported genetic variation for low P tolerance in WA pearl millet. Beggi et al. (unpublished), Brück et al. (2003) and Faye et al.

(2006) reported genotypic differences for pearl millet in root and shoot parameters in pot experiments, fields with P deficiency and in hydroponic conditions, respectively. In their work, Beggi et al. (unpublished) showed genotypic differences for P uptake and P utilization efficiencies in a wide range of pearl millet open pollinated varieties. Plants generally respond to P deficiency by partitioning assimilates preferentially to the roots leading to an increased root to shoot ratio under LP (Smith et al., 1990; Kirk et al., 1998; Vance et al., 2003; Corrales et al., 2007). We observed increased root to shoot ratio (RS) and P utilization efficiency (PUTE) under LP in our study implying that low P tolerance was mainly achieved by improved P uptake by root exploration through proliferation and extension as well as by the more efficient use of internal P (Vance et al., 2003; Lynch and Ho, 2005; Ramaekers et al., 2010; Wang et al., 2010). Rose et al. (2011) hypothesized that genotypes with a higher P uptake efficiency would suffer less P stress and this would lead to a lower P utilization efficiency. This means that the two traits are confounded and hence it is difficult to tell which one is more important. Similar to their study, we observed a negative correlation between total P in shoots (PS) and P utilization efficiency (PUTE) suggesting that the two traits cannot be selected together at an early growth stage. This is contrary to the results obtained from mature plants under field conditions where P uptake (PBM) and P utilization efficiency (PUTE) were not correlated, although P utilization efficiency (PUTE) was also found to be higher under LP in the field experiment. The confounding effects of P uptake on P utilization could also be aggravated under pot conditions due to a lower available P in the soil for uptake as a result of the limited soil exploration allowed by the pots. Although most studies have indicated shoot growth to be an appropriate parameter for low P tolerance at early growth stage (Batten, 1986; Fageria and Baligar, 1997; Osborne and Rengel, 2002; Ozturk et al., 2005; Beggi and Bürkert, unpublished), Ozturk et al. (2005) correctly points out the need to separate carbon partitioning from low P tolerance. This would be mainly important especially in cases where there is a high correlation between HP and LP and presence of genetic variation under HP as well. We observed high genetic correlation between HP and LP for shoot dry biomass (SDB). Since low P tolerance is defined as SDB_{LP}/SDB_{HP}, the correlation between shoot dry biomass and low P tolerance could just be an autocorrelation. Another limitation of shoot dry biomass is that it is destructive in nature. Simple non destructive traits like seedling vigor (SV) or height (HT) which also had high positive correlations with stem diameter (SD), root dry biomass (RDB), shoot dry biomass (SDB) and root to shoot ratio (RS) would offer a working alternative in early growth stage selection for low P tolerance especially for breeding trials where a lot of genotypes are included. Both these traits (seedling vigor; SV and height; HT) had relatively higher repeatability estimates compared to most of the other traits measured under pot conditions. We also observed a relatively better correlation between root dry biomass (RDB), root to shoot ratio (RS) and GY relative to shoot dry biomass (SDB) despite the high correlation among the three traits (RDB, SDB and RS) and this indicates the importance of enhanced P uptake under LP. P uptake has been shown to be more important than P utilization under limited P in wheat, rice, common bean, pigeon pea, *Brassica napus* and pearl millet (Manske et al., 2001; Wissuwa and Ae, 2001; Fujita et al., 2004; Beebe et al., 2006; Panigrahy et al., 2009; Zhang et al., 2009; Wang et al., 2010; Beggi and Bürkert, unpublished). Despite its importance for biomass and grain production under LP, P uptake would enhance continued soil mining of P (Henry et al., 2010) and as so, selection for P utilization would be more necessary especially for WA soils which are already depleted. However, selection for P utilization efficiency at an early stage has a limitation of having a lower P source at later stages and consequent negative effects on nutritional quality of grain and crop residues (Batten, 1986; Maroko et al., 1999). P uptake efficiency would therefore be necessary at this stage to ensure enough P accumulation for later plant development stages.

4.4. Selection for low P tolerance based on P related secondary traits is possible

We observed significant correlations between early growth stage morphological traits as well as P-parameters assessed in pot conditions under LP, those measured in field conditions under LP and GY from independent environments under LP implying that most of the traits measured could be used as secondary traits for high grain yield selection under LP. Grain yield is a complex trait with reduced heritability ($w^2 = 0.59$ under LP in the current study) especially under stress and this makes it difficult to select for this trait without multi-environment field trials which are also expensive (Passioura, 2010; Passioura and Angus, 2010; Passioura, 2012; Rebetzke et al., 2012). The use of secondary traits with higher heritabilities especially at early growth stages in combination with grain yield could therefore expedite the breeding process. We observed positive response to selection for most morphological and P-parameters measured under both field and pot conditions as trait *per se* as well as in a GY and trait index. This is an encouraging finding as it suggests that it is possible to increase pearl millet grain yield by selecting for P use efficiency. For instance, direct selection for grain yield under LP at 10% selection intensity resulted in 54.2 gm^{-2} yield increase over the grand mean (53.8 gm^{-2}) which means that grain yield was doubled. Being able to double pearl millet grain yield would significantly contribute to food security within WA where some of the world's most poor live (Haussmann et al., 2012). We also noted that response to selection increased with index selection as compared to trait *per se* selection. In the current study, several traits in an index selection especially under field conditions gave responses to selection which were numerically higher than grain yield *per se* selection. For instance, index selection combining grain yield with seedling vigor, stover weight or P uptake under field conditions gave the highest responses to selection (60.9, 70.3 and 70.8 gm^{-2}, respectively, above the grand mean) as compared to grain yield as trait *per se* selection (54.2 gm^{-2} above grand mean) under LP. These traits had repeatability estimates of 0.65, 0.61 and 0.59, respectively, under LP. Although the repeatability estimates did not differ much from that of grain yield as a trait *per se* (0.59), the efficiency of selection was improved in a grain yield and trait index selection resulting in numerically higher selection responses than in grain *per se* selection. Banziger et al. (2000), Lafitte et al. (2003) and Abdel-Ghani et al. (2013) also showed that combining selection for grain yield with secondary traits could increase selection efficiency in maize under low nitrogen conditions. Generally, the repeatability estimates observed in our study were lower than those reported by Abdel-Ghani et al. (2013) in maize inbred lines under low nitrogen and this is a direct consequence of the harsh growing conditions associated with the WA Sahel which further reinforces the challenge of developing a suitable selection framework for low P in WA.

In the current study also, the correlation of grain yields under LP across locations was relatively high. This implies that genotype by location interaction was lower and this would mean that selection for grain yield under LP as a trait *per se* can be done in fewer locations. However, it should be noted that the current study is based on inbred lines with variable degrees of inbreeding depression. According to the quantitative genetic theory, the genetic coefficient of variation is expected to be about four times more in inbred lines than that of testcrosses (Smith, 1986; Miedaner et al., 2014). This therefore culminates into a higher heritability in inbred lines compared to more heterozygous or heterogenous populations. In Gemenet et al. (2014), we showed that genetic variation reduced by about half in testcrosses compared to inbred lines, and with it, a

reduced heritability for grain yield under LP in testcrosses. The findings from the current study should therefore be validated further on a testcross or a population panel.

The positive response to selection as traits *per se* and index selection of early growth traits measured under pot conditions imply that selection at seedling stage in pots under LP for field performance under LP and subsequent GY production is possible. However, it should also be noted that response to selection as well as repeatability estimates were better under field conditions as compared to pot conditions. Furthermore, most of the pot-measured traits had a lower consistency of selection response measured by the standard deviation. This can be explained by the fact that the crucial role of the soil environment on plant growth in the field cannot be simulated under pot conditions (Whitmore and Whalley, 2009) due to the heterogeneity in the fields. Pot experiments have limitations of reduced soil quantity and consequently, limited water and nutrients to the plant (Passioura, 2006; Poorter et al., 2012). In addition, pots also impede root growth. Pearl millet has been shown to have a lot of crown roots and an enhanced rooting angle to exploit the soil depth both vertically and horizontally to a depth of 2 m at later stages (Bruck et al., 2003). We also observed differences on the relationship between P uptake and P utilization efficiencies at early growth stage and in mature plants despite P utilization efficiency (PUTE) in the pots having positive relationships with P uptake (PBM) and P utilization efficiency (PUTE) in the field. Similar to Rose et al. (2011) also working on early growth stage rice plants, we observed that P uptake and P utilization efficiencies were negatively correlated at early growth stage whereas there was no correlation between the two traits at mature plant stage as also reported by Parentoni and Souza (2008) in maize. This could mean that there are different P fluxes in young and mature plants. Schultz and French (1978) observed that shoot P concentration declined with maturity and that only data from the same developmental stage could be compared. Phenotyping for phosphorus efficiency at early growth stages under pot environments rather than field evaluations should therefore be determined by the objective of the phenotyping, the heritability of the trait and the logistical considerations of collecting data such as economic considerations, time, selection intensity, among others (Cobb et al., 2013; Araus and Cairns, 2014).

5. Conclusions

Although difficulties were encountered to achieve a strong P effect especially under field conditions, important deductions can be made based on the current results. Genetic variation for low P tolerance exists in WA pearl millet inbred lines at both early growth and mature plant stages. P utilization efficiency increased under LP although P uptake efficiency was more important for grain production. There is need to improve both P uptake and P utilization efficiencies, with the optimal balance between the two depending on soil type, soil P reserves and soil fertility management practices common in the breeding program's target region. P utilization efficiency as a trait is confounded by P uptake at early growth stage and by P uptake and harvest index at mature plant stage. Internal P use efficiency could therefore be improved by selecting for a lower P concentration in grain as well as a lower ratio between P harvest index and grain harvest index at mature plant stage. At early growth stage, selecting for P utilization efficiency is not recommended so as to avoid effects of a lower P source at later developmental stages. Most morphological and P-parameters measured could be used as secondary traits for higher grain yield under LP but response to selection based on early growth traits was lower. There is however need to validate these results in testcrosses and/or population varieties to be able to identify the possible effect of variable degrees of inbreeding in the inbred line panel on the relationship of traits like height, seedling vigor, among others and grain yield. To be able to make pearl millet production systems in WA more productive and sustainable, breeding activities for low phosphorus tolerance should be integrated with other system-oriented research such as nutrient cycling, intercropping or rotations with legumes, better crop-tree-livestock integration, and modest applications of locally available rock phosphate.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fcr.2014.11.001.

References

Abdel-Ghani, A.H., Kumar, B., Reyes-Matamoros, J., Gonzalez-Portilla, P.J., Jansen, C., Martin, J.P.S., Lee, M., Lübberstedt, T., 2013. Genotypic variation and relationships between seedling and adult plant traits in maize (*Zea mays* L.) inbred lines grown under contrasting nitrogen levels. Euphytica 189, 123–133, http://dx.doi.org/10.1007/s10681-012-0759-0.

Ai, P., Sun, S., Zhao, J., Fan, X., Xin, W., Guo, Q., Yu, L., Shen, Q., Wu, P., Miller, A.J., Xu, G., 2009. Two rice phosphate transporters, OsPht1;2 and OsPht1;6, have different functions and kinetic properties in uptake and translocation. Plant J. 57, 798L 809.

Araújo, A.P., Teixeira, M.G., 2003. Nitrogen and phosphorus harvest indices of common bean cultivars: implications for yield quantity and quality. Plant Soil 257, 425–433, http://dx.doi.org/10.1023/A:1027353822088.

Araus, J.L., Cairns, J.E., 2014. Field high-throughput phenotyping: the new crop breeding frontier? Trends Plant Sci. 19 (1), 52–61.

Banziger, M., Edmeades, G.O., Beck, D., Bellon, M., 2000. Breeding for Drought and Nitrogen Stress Tolerance in Maize: From Theory to Practice. CIMMYT, Mexico.

Barry, D., Miller, M., 1989. Phosphorus nutritional requirement of maize seedlings for maximum yield. Agron. J. 81, 95L 99.

Bationo, A., Christianson, B.C., Klaij, M.C., 1993. The effect of crop residue and fertilizer use on pearl millet yields in Niger. Fertil. Res. 34, 251L 258.

Batten, G.D., 1986. The uptake and utilization of phosphorus and nitrogen by diploid, tetraploid and hexaploid wheats (*Triticum* spp.). Ann. Bot. 58, 49–59.

Batten, G.D., Slack, K., 1990. Grain development in wheat (*Triticum aestivum*) ears cultured in media with different concentrations of phosphorus and sucrose. In: van Beusichem, M.L. (Ed.), Plant Nutrition—Physiology and Applications. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 185–187.

Bayuelo-Jiménez, J.S., Ochoa-Cadavid, I., 2014. Phosphorus acquisition and internal utilization efficiency among maize landraces from the central Mexican highlands. Field Crops Res. 156, 123L 134.

Beebe, S.E., Rojas-Pierce, M., Yan, X., Blair, M.W., Pedraza, F., Mũnoz, F., Tohme, J., Lynch, J.P., 2006. Quantitative trait loci for root architecture traits correlated with phosphorus acquisition in common bean. Crop Sci. 46, 413L 423.

Bieleski, R.L., 1976. Phosphate pools, phosphate transport and phosphate availability. Annu. Rev. Plant Physiol. 24, 225L 252.

Blum, A., 2011. Plant Breeding for Water-limited Environments. Springer, New York, http://dx.doi.org/10.1007/978-1-4419-7491-4.

Brück, H., Sattelmacher, B., Payne, W.A., 2003. Varietal differences in shoot and rooting parameters of pearl millet on sandy soils in Niger. Plant Soil 251, 175L 185.

Buerkert, A., Bationo, A., Piepho, H-P., 2001. Efficient phosphorus application strategies for increased crop production in sub-Saharan West Africa. Field Crops Res. 72, 1L 15.

Burleigh, S.H., Cavagnaro, T., Jakobsen, I., 2002. Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition. J. Exp. Bot. 53, 1591–1601.

Calderon-Vazquez, C., Ibarra-Laclette, E., Caballero-Perez, J., Herrera-Estrella, L., 2008. Transcript profiling of *Zea mays* roots reveals gene responses to phosphate deficiency at the plant- and species-specific levels. J. Exp. Bot. 59 (9), 2479–2497, http://dx.doi.org/10.1093/jxb/ern115.

Cichy, K.A., Snapp, S.S., Blair, M.W., 2008. Plant growth habit, root architecture traits and tolerance to low soil phosphorus in an Andean bean population. Euphytica 165, 257–268, http://dx.doi.org/10.1007/s10681-008-9778-2.

Cobb, J.N., DeClerck, G., Greenberg, A., Clark, R., McCouch, S., 2013. Next-generation phenotyping: requirements and strategies for enhancing our understanding of genotype phenotype relationships and its relevance to crop improvement. Theor. Appl. Genet. 126, 867–887, http://dx.doi.org/10.1007/s00122-013-2066-0.

Cooper, M., Delacy, I.H., Basford, K.E., 1996. Relationships among analytical methods used to analyze genotypic adaptation in multi-environment trials. In: Cooper, M., Hammer, G.L. (Eds.), Plant Adaptation and Crop Improvement. CAB Int., Wallingford, UK, pp. 193–224.

Corrales, I., Amenós, M., Poschenrieder, C., Barceló, J., 2007. Phosphorus efficiency and root exudates in two contrasting tropical maize varieties. J. Plant Nutr. 30, 887–900.

Doumbia, M.D., Sidibé, A., Bagayoko, A., Diarra, M.A., Bationo, A., Kablan, R.A., Yost, R.S., Hossner, L.R., Hons, F.M., 2003. Recommandations specifiques díengrais: calibration et validation du module phosphore du modèle NuMaSS. Afr. Crop Sci. J. 11, 17–26.

Fageria, N.K., Baligar, V.C., 1997. Upland rice genotypes evaluation for phosphorus use efficiency. J. Plant Nutr. 20, 499L 509.

Fang, Z.Y., Shao, C., Meng, Y.J., Wu, P., Chen, M., 2009. Phosphate signaling in *Arabidopsis* and *Oryza sativa*. Plant Sci. 176, 170–180.

Faye, I., Diouf, O., Guissé, A., Sène, M., Diallo, N., 2006. Characterizing root responses to low phosphorus in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Agron. J. 98, 1187–1194.

Fitter, A.H., Hay, R.K.M., 2002. Environmental Physiology of Plants. Academic Press, London.

Fujita, K., Kai, Y., Takayanagi, M., El-Shemy, H., Adu-Gyamfi, J.J., Mohapatr, P.K., 2004. Genotypic variability of pigeon pea in distribution of photosynthetic carbon at low phosphorus level. Plant Sci. 166, 641–649 [42].

Gemenet, D.C., Hash, C.T., Sy, O., Zangre, R.G., Sanogo, M.D., Leiser, W.L., Parzies, H.K., Haussmann, B.I.G., 2014. Pearl millet inbred and testcross performance under low phosphorus in West Africa. Crop Sci. (in press).

Gerloff, S., 1977. Plant efficiencies in the use of N, P and K. In: Wright, M.J. (Ed.), Plant Adaptation to Mineral Stress in Problem Soils. Cornell Univ. Press, New York, pp. 161–174.

Hajabbasi, M.A., Schumacher, T.E., 1994. Phosphorus effects on root growth and development in two maize genotypes. Plant Soil 158, 39–46.

Hammond, J.P., Broadley, M.R., White, P.J., 2004. Genetic responses to phosphorus deficiency. Ann. Bot. 94, 323–332, http://dx.doi.org/10.1093/aob/mch156.

Hammond, J.P., White, P.J., 2008. Sucrose transport in the phloem: integrating root responses to phosphorus starvation. J. Exp. Bot. 59, 93–109.

Haussmann, B.I.G., Rattunde, H.F., Weltzien-Rattunde, E., Traore, P.S.C., vom Brocke, K., Parzies, H.K., 2012. Breeding strategies for adaptation of pearl millet and sorghum to climate variability and change in West Africa. J. Agron. Crop Sci. 198, 327–339, http://dx.doi.org/10.1111/j.1439-037X.2012.00526.x.

Henry, A., Rosas, J.C., Beaver, J.S., Lynch, J.P., 2010. Multiple stress response and below ground competition in multilines of common bean (*Phaseolus vulgaris* L.). Field Crops Res. 117, 209–218.

Holford, I.C., 1997. Soil phosphorus: its measurement, and its uptake by plants. Austr. J. Soil Res. 35, 227–240.

Houle, D., Govindaraju, D.R., Omholt, S., 2010. Phenomics: the next challenge. Nat. Rev. Genet. 11, 855L 866.

Hurrell, R.F., Reddy, M.B., Juillerat, M-A., Cook, J.D., 2003. Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. Am. J. Clin. Nutr. 77, 1213L 1219.

Ismail, A.M., Heuer, S., Thomson, J.T., Wissuwa, M., 2007. Genetic and genomic approaches to develop rice germplasm for problem soils. Plant Mol. Biol. 65, 547L 570.

Jones, G.P.D., Blair, G.J., Jessop, R.S., 1989. Phosphorus efficiency in wheat—a useful selection criterion? Field Crops Res. 21, 257–264, http://dx.doi.org/10.1016/0378-4290(89)90007-5.

Kirk, G.J.D., George, T., Courtois, B., Senadhira, D., 1998. Opportunities to improve phosphorus efficiency and soil fertility in rainfed lowland and upland rice ecosystems. Field Crops Res. 56, 73L 92.

Kochian, L.V., 2012. Plant nutrition: rooting for more phosphorus. Nature 488, 466–467, http://dx.doi.org/10.1038/488466a.

Lafitte, R., Blum, A., Atlin, G., 2003. Using secondary traits to help identify drought-tolerant genotypes. In: Fischer, K.S., Lafitte, R., Fukai, S., Atlin, G., Hardy, B. (Eds.), Breeding Rice for Drought-prone Environments. IRRI, Los Banos, Philippines, pp. 37–48.

Lambers, H., Shane, M.W., Cramer, M.D., Pearse, S.J., Veneklaas, E.J., 2006. Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. Ann. Bot. 98, 693–713.

Leiser, W.L., Rattunde, H.F.W., Piepho, H.P., Weltzien, E., Diallo, A., Melchinger, A.E., Parzies, H.K., Haussmann, B.I.G., 2012. Selection strategy for sorghum targeting phosphorus limited environments in West Africa: analysis of multi-environment experiments. Crop Sci. 52, 2517L 2527.

Leiser, W.L., Rattunde, H.F.W., Weltzien, E., Haussmann, B.I.G., 2014a. Phosphorus uptake and use efficiency of diverse West and Central African sorghum genotypes under field conditions in Mali. Plant Soil, http://dx.doi.org/10.1007/s11104-013-1978-4.

Leiser, W.L., Rattunde, H.F.W., Piepho, H.-P., Weltzien, E., Diallo, A., Toure, A., Haussmann, B.I.G., 2014b. Selection criteria for enhancing sorghum yield in phosphorous-limited production systems: trait relationships with grain yield in independent low-P environments. Crop Sci. (in press).

Lynch, J., 1995. Root architecture and plant productivity. Plant Physiol. 109, 7–13.

Lynch, J.P., Ho, M.D., 2005. Rhizoeconomics. Carbon costs of phosphorus acquisition. Plant Soil 269, 45–56.

Manga, V.K., Saxena, M.B.L., 1988. Association of root and related traits at different growth stages in pearl millet. Crop Improve. 15, 105L 106.

Manschadi, A.M., Kaul, H-P., Vollmann, J., Eitzinger, J., Wenzel, W., 2014. Developing phosphorus-efficient crop varieties—an interdisciplinary research framework. Field Crops Res. 162, 87–98.

Manske, G.G.B., Ortiz-Monasterio, J.I., van Ginkel, M., Gonzaílez, R.M., Fischer, R.A., Rajaram, S., Vlek, P.L.G., 2001. Importance of P uptake efficiency versus P utilization for wheat yield in acid and calcareous soils in Mexico. Eur. J. Agron. 14, 261–274, http://dx.doi.org/10.1016/S1161-0301(00)00099-X.

Manske, G.G.B., Ortiz-Monasterio, J.I., Van Ginkel, M., González, R.M., Rajaram, S., Molina, E., Vlek, P.L.G., 2000. Traits associated with improved P-uptake efficiency in CIMMYT's semidwarf spring bread wheat grown on an acid Andisol in Mexico. Plant Soil 221, 189–204.

Manu, A., Bationo, A., Geiger, S.C., 1991. Fertility status of selected millet producing soils of West Africa with emphasis on phosphorus. Soil Sci. 152, 315L 320.

Maroko, J.B., Buresh, R.J., Smithson, P.C., 1999. Soil phosphorus fractions in unfertilized fallow-maize systems on two tropical soils. Soil Sci. Soc. Am. J. 63, 320–326.

Miedaner, T., Schwegler, D.D., Wilde, P., Reif, J.C., 2014. Association between line per se and testcross performance for eight agronomic and quality traits in winter rye. Theor. Appl. Genet. 127, 33–41.

Mir, R.R., Zaman-Allah, M., Sreenivasulu, N., Trethowan, R., Varshney, R.K., 2012. Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. Theor. Appl. Genet., http://dx.doi.org/10.1007/s00122-012-1904-9.

Oberstein, M., Peñuelas, J., Ciais, P., van der Velde, M., Janssens, I.A., 2013. The phosphorus trilemma. Nat. Geosci. 6, 897–898.

Osborne, L.D., Rengel, Z., 2002. Screening cereals for genotypic variation in efficiency of phosphorus uptake and utilization. Austr. J. Agric. Res. 53, 295L 303.

Ozturk, L., Eker, S., Torun, B., Cakmak, I., 2005. Variation in phosphorus efficiency among 73 bread and durum wheat genotypes grown in a phosphorus-deficient calcareous soil. Plant Soil 269, 69–80, http://dx.doi.org/10.1007/s11104-004-0469-z.

Panigrahy, M., Rao, D.N., Sarla, N., 2009. Molecular mechanisms in response to phosphate starvation in rice. Biotechnol. Adv. 27, 389L 397.

Parentoni, S., de Souza Jr., C., de Carvalho Alves, V., Gama, E., Coelho, A., de Oliveira, A., Guimaraes, P., Guimaraes, C., Vasconcelos, M., Pacheco, C.A.P., Meirelles, W.F., de Magalhaes, J.V., Moreira Guimaraes, L.J., da Silva, A.R., Ferreira Mendes, F., Schaffert, R.E., 2010. Inheritance and breeding strategies for phosphorus efficiency in tropical maize (*Zea mays* L.). Maydica 55, 1–15.

Parentoni, S.N., Souza Jr., C.L., 2008. Phosphorus acquisition and internal utilization efficiency in tropical maize genotypes. Pesq. Agrop. Bras. 43, 893–901.

Passioura, J.B., 2006. The perils of pot experiments. Funct. Plant Biol. 33, 1075L 1079.

Passioura, J.B., 2010. Scaling up: the essence of effective agricultural research. Funct. Plant Biol. 37, 585L 591.

Passioura, J.B., 2012. Phenotyping for drought tolerance in grain crops: when is it useful to breeders? Funct. Plant Biol. 39, 851–859, 24.

Passioura, J.B., Angus, J.F., 2010. Improving productivity of crops in water-limited environments. Adv. Agron. 106, 37–75, 23.

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

Plenet, D., Etchebest, S., Mollier, A., Pellerin, S., 2000. Growth analysis of maize field crops under phosphorus deficiency. I. Leaf growth. Plant Soil 223, 117–130.

Poorter, H., Bühler, J., van Dusschoten, D., Climent, J., Postma, J.A., 2012. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. Funct. Plant Biol. 39, 839–850.

Pucher, A., Hogh-Jensen, H., Gondah, J., Hash, C.T., Haussmann, B.I.G., 2014. Micronutrient density and stability in West African pearl millet—potential for biofortification. Crop Sci. 54, 1709L 1720.

Raboy, V., 2009. Approaches and challenges to engineering seed phytate and total phosphorus. Plant Sci. 177, 281–296, http://dx.doi.org/10.1016/j.plantsci.2009.06.012.

Ramaekers, L., Remans, R., Rao, I.M., Blair, M.W., Vanderleyden, J., 2010. Strategies for improving phosphorus acquisition efficiency of crop plants. Field Crops Res. 117, 169–176.

Rebafka, F.P., Bationo, A., Marschner, H., 1993. Phosphorus seed coating increases phosphorus uptake, early growth and yield of pearl millet (*Pennisetum glaucum* (L.) R. Br.) grown on an acid sandy soil in Niger, West Africa. Fertil. Res. 35, 151–160.

Rebetzke, G.J., Chenu, K., Biddulph, B., Moeller, C., Deery, D.M., Rattey, A.R., Bennett, D., Barrett-Lennard, E.G., Mayer, J.E., 2013. A multisite managed environment

facility for targeted trait and germplasm phenotyping. Funct. Plant Biol. 40, 1–13, http://dx.doi.org/10.1071/FP12180.

Rebetzke, G.J., van Herwaarden, A., Biddulph, B., Chenu, K., Moeller, C., Richards, R.A., Rattey, A., 2012. Protocols for experimental plot sampling, handling and processing of cereal experiments–standardised methods for use in field studies. In: PrometheusWiki. CSIRO Publishing, Collingwood, Available at: ⟨http://prometheuswiki.publish.csiro.au/tiki-index.php⟩.

Reynolds, M.P., Trethowan, R.M., 2007. Physiological interventions in breeding for adaptation to abiotic stress. In: Spiertz, J.H.J., Struik, P.C., van Laar, H.H. (Eds.), Scale and Complexity in Plant Systems Research, Gene–Plant–Crop Relations. Wageningen UR Frontis Series, vol. 21. Springer, pp. 129–146.

Rose, T.J., Pariasca-Tanaka, J., Rose, M.T., Fukuta, Y., Wissuwa, M., 2010. Genotypic variation in grain phosphorus concentration, and opportunities to improve P-use efficiency in rice. Field Crops Res. 119, 154–160, http://dx.doi.org/10.1016/j.fcr.2010.07.004.

Rose, T.J., Rose, M.T., Pariasca-Tanaka, J., Heuer, S., Wissuwa, M., 2011. The frustration with utilization: why have improvements in internal phosphorus utilization efficiency in crops remained so elusive? Front. Plant Sci., http://dx.doi.org/10.3389/fpls.2011.00073.

Schultz, J., French, R., 1978. The mineral content of cereals, grain legumes and oilseed crops in South Australia. Austr. J. Exp. Agric. 18, 579L 585.

Scott-Wendt, J., 1988. Variability in pearl millet (*Pennisetum americanum*) fields in semi-arid West Africa. Arid Soil Res. Rehabil. 2, 49–58.

Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W., Zhang, F., 2011. Phosphorus dynamics: from soil to plant. Plant Physiol. 156, 997L 1005.

Smith, O.S., 1986. Covariance between line per se and testcross performance. Crop Sci. 26, 540L 543.

Smith, S.W., Jackson, W.A., Van den Berg, P.J., 1990. Internal phosphorus flows during development of phosphorus stress in *Stylosanthes hamata*. Austr. J. Plant Physiol. 17, 451L 464.

Stoorvogel, J.J., Smaling, E.M.A., Janssen, B.H., 1993. Calculating soil nutrient balances in Africa at different scales. Fertil. Res. 35, 227–235, http://dx.doi.org/10.1007/BF00750641.

Valluru, R., Vadez, V., Hash, C.T., Karanam, P., 2010. A minute phosphorus application contributes to a better establishment of pearl millet (*Pennisetum glaucum* (L.) R. Br.) seedling in phoshorus deficient soils. Soil Use Manage. 26, 36–43.

Vance, C.P., Uhde-Stone, C., Allan, D.L., 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol. 157, 423–447.

VDLUFA, 2011. Umweltanalytik. VDLUFA-Verl, Darmstadt.

Wang, X., Shen, J., Liao, H., 2010. Acquisition or utilization, which is more critical for enhancing phosphorus efficiency in modern crops? Plant Sci. 179, 302–306, http://dx.doi.org/10.1016/j.plantsci.2010.06.007.

White, P.J., Veneklaas, E.J., 2012. Nature and nurture: the importance of seed phosphorus content. Plant Soil 357, 1–8, http://dx.doi.org/10.1007/s11104-012-1128-4.

Whitmore, A.P., Whalley, W.R., 2009. Physical effects of soil drying on roots and crop growth. J. Exp. Bot. 60, 2845–2857.

Wissuwa, M., Ae, N., 2001. Genotypic variation for tolerance to phosphorus deficiency in rice and the potential for its exploitation in rice improvement. Plant Breed. 120, 43–48, http://dx.doi.org/10.1111/j.1439-0523.2001.tb01698.x.

Wissuwa, M., Mazzola, M., Picard, C., 2009. Novel approaches in plant breeding for rhizosphere-related traits. Plant Soil 321, 409–430.

Wissuwa, M., Yano, M., Ae, N., 1998. Mapping of QTLs for phosphorus-deficiency tolerance in rice (*Oryza sativa* L.). Theor. Appl. Genet. 97, 777–783, http://dx.doi.org/10.1007/s001220050955.

Yang, X.J., Finnegan, P.M., 2010. Regulation of phosphate starvation responses in higher plants. Ann. Bot. 105, 513–526, http://dx.doi.org/10.1093/aob/mcq015.

Yaseen, M., Malhi, S.S., 2010. Selection criteria for genotypes and identification of plant characteristics for phosphorus-efficient wheat. Commun. Soil Sci. Plant Anal. 41, 679–695.

Zhang, H., Huang, Y., Ye, X., Shi, L., Xu, F., 2009. Genotypic differences in phosphorus acquisition and the rhizosphere properties of *Brassica napus* in response to low phosphorus stress. Plant Soil 320, 91–102.