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Association analysis of low-phosphorus tolerance in West African pearl millet using DArT markers

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Abstract Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a food security crop in the harshest agricultural regions of the world. While low soil phosphorus (P) availability is a big constraint on its production, especially in West Africa (WA), information on genomic regions responsible for low-P tolerance in pearl millet is generally lacking. We present the first report on genetic polymorphisms underlying several plant P-related parameters, flowering time (FLO) and grain yield (GY) under P-limiting conditions based on 285 diversity array technology markers and 151 West African pearl millet inbred lines phenotyped in six environments in WA under both high-P and low-P conditions. Nine markers were significantly associated with P-related traits, nine markers were associated with FLO, whereas 13 markers were associated with GY each explaining between 5.5 and 15.9 % of the observed variation. Both constitutive and adaptive associations were observed for FLO and GY, with markers *PgPb11603* and *PgPb12954* being associated with the most stable effects on FLO and GY, respectively, across locations. There were a few shared polymorphisms between traits, especially P-efficiency-related traits and GY, implying possible colocation of genomic regions responsible for these traits. Our findings help bridge the gap between quantitative and molecular methods of studying complex traits like low-P tolerance in WA. However, validation of these markers is necessary to

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determine their potential applicability in marker-assisted selection programs targeting low-P environments, which are especially important in WA where resource-poor farmers are expected to be the hardest hit by the approaching global P crisis.

Keywords Low phosphorus availability · West Africa · Marker–trait association · DArT markers

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br., syn. *Cenchrus americanus* (L.) Morrone], (2n = 2x = 14) is the sixth most important global cereal crop. It is produced as a rain-fed grain and fodder crop in the hottest, driest regions of sub-Saharan Africa and the Indian subcontinent (Andrews and Kumar 1992; Sehgal et al. 2012). It is the most important cereal for food security in the West African Sudano–Sahelian belt, where it is grown as a staple crop by some of the poorest people of the world (Haussmann et al. 2012). Pearl millet ensures food security by providing both calories and essential micronutrients more reliably than any other cereal under dryland conditions in these regions (Goswami et al. 1969; Sawaya et al. 1984; Stich et al. 2010; Sehgal et al. 2012). Despite its importance, its production within this region is hampered by erratic rainfall, acidic soils and low soil fertility among other production constraints (Brück et al. 2003). The importance of low soil phosphorus (P) availability on yield reduction is well documented within the region (Bationo et al. 1986, 1990; Bationo and Mokwunye 1991; Payne et al. 1991; Rebafka et al. 1994; Muehlig-Versen et al. 2003). Given the economic constraints related to fertilizer access within this region, it is therefore imperative that plant breeders put more efforts into developing pearl millet varieties that are tolerant to low soil P conditions (Hash et al. 2002). Recently, genetic variation for performance under low-P conditions was reported in West African pearl millet open-pollinated varieties, inbred lines and testcrosses, where direct selection under low-P conditions was shown to have potential for improving pearl millet grain yield in P-limited environments (Gemenet et al. 2014, 2015a, 2015b). Given the difficulties associated with field evaluation for low-P tolerance due to numerous interactions with drought and other soil properties in WA, marker-assisted selection would assist in shortening the breeding process targeting low-P environments.

Marker-assisted selection has the potential to expedite the breeding process, but requires proper estimation of the positions and effects of quantitative trait loci (QTLs; Stich et al. 2008; Supriya et al. 2011). Since the 1990s, several reports about QTL mapping P-deficiency tolerance have been published and QTLs for P-deficiency tolerance-related traits have been mapped in several crops including maize (Reiter et al. 1991; Chen et al. 2009; Li et al. 2010; Zhang et al. 2014), rice (Wissuwa et al. 1998, 2002), soybean (Li et al. 2005) and common bean (Yan et al. 2001; Beebe et al. 2006). Although the prospects of marker-assisted selection to enhance performance under low-P conditions in 'orphan crops' have been clearly outlined by Hash et al. (2002), not much previous effort has gone into identifying genomic regions responsible for P-deficiency tolerance-related traits in pearl millet. Most efforts in biparental QTL mapping in pearl millet have been directed toward drought tolerance (Yadav et al. 2002, 2004; Sehgal et al. 2012) and downy mildew resistance (Jones et al. 1995, 2002; Hash et al. 1995; Hash and Witcombe 2001; Breese et al. 2002; Gulia et al. 2007) and demonstrations of the effectiveness of marker-assisted selection for these traits (Bidinger et al. 2005; Hash et al. 2006a, b; Khairwal and Hash 2007; Kholová et al. 2010a, b; Nepolean et al. 2009; Serraj et al. 2005). Association mapping utilizes ancestral recombination in natural populations to overcome the limitations associated with classical linkage mapping of reduced resolution of biparental mapping populations due to small population sizes and modest degrees of recombination (Flint-Garcia et al. 2003, 2005: Kraakman et al. 2004; Stich et al. 2008). Using association mapping, Leiser et al. (2014) and Hufnagel et al. (2014) have detected several genomic regions in sorghum associated with various low-P tolerance traits at different stages of crop maturity.

The ability to identify useful phenotype–genotype associations through association analysis can be limited by several factors including population structure leading to high false positives, extended linkage disequilibrium (LD) blocks resulting from selective events or stochastic probabilities, and epistasis as well as rare causal alleles that require large populations for detection (Chan et al. 2010) and/or are better addressed using targeted biparental mapping populations. Having been domesticated in the Sahelian zone

of West Africa (WA; Harlan et al. 1976; Tostain 1992; Mariac et al. 2006; Manning 2011), cultivated pearl millet displays tremendous phenotypic variability for traits such as flowering time, panicle length, grain and stover characteristics, tolerance to drought, pests and diseases, as well as nutritional value (Bhattacharjee et al. 2007; Stich et al. 2010; Bashir et al. 2014a, 2014b; Pucher et al. 2014, 2015). This can be attributed to genetic differentiation as a consequence of many factors including local adaptation, selection and genetic drift, which can lead to non-random distribution of important agronomic traits (Hedrick 2005; Lewis 2010). Despite this large variation, it has been established that neither country of origin nor agroecological zone shows a clear differentiation of pearl millet landrace genotypes (probably due to their highly cross-pollinated breeding behavior and robust wind-borne pollen); but rather, populations are differentiated into subgroups based on their parentage and/or similar agronomic traits (Tostain et al. 1987; Oumar et al. 2008; Stich et al. 2010; Bashir et al. 2015).

Due to their high polymorphic information content, simple sequence repeat markers (SSRs) have been applied as markers of choice in most pearl millet genetic diversity studies (Vigouroux et al. 2005; Mariac et al. 2006; Kapila et al. 2008; Lewis 2010; Stich et al. 2010; Gupta et al. 2012; Nepolean et al. 2012). Only one published study has applied diversity array technology (DArT) markers in pearl millet genetic diversity analysis and/or linkage map saturation (Supriya et al. 2011; Kholová et al. 2012), despite it being shown that their cost per data point is about one-tenth that of SSRs (Xia et al. 2005). A pearl millet DArT platform has been developed in the M.S. Swaminathan Center of Excellence in Genomics at the Indian headquarters of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT; Supriya et al. 2011). DArT is a cost-effective, solid-state platform, hybridization-based marker technology offering high multiplexing without prior sequence information (Jaccoud et al. 2001; Wenzl et al. 2004; Supriya et al. 2011) and is the least expensive way to achieve genome-wide marker coverage without extensive DNA sequence data. Since their initial development in rice, DArT markers have gained importance in genetic mapping, genotyping and diversity assessment in many important crops such as barley (Wenzl et al. 2004), cassava (Hurtado et al. 2008), pigeon pea (Yang et al. 2006), wheat (Zhang et al. 2011), sorghum (Mace et al. 2008), oat (Tinker et al. 2009), rye (Bolibok-Bragoszewska et al. 2009), *Triticum monococcum* (Jing et al. 2009) and white lupin (Raman et al. 2014).

The main objective of the present study therefore was to identify genetic regions underlying quantitative traits under low-P conditions such as P concentration in stover (PCS), P concentration in grain (PCG), P-uptake efficiency (PBM), P-utilization efficiency (PUE), time to 50 % flowering (FLO) and grain yield (GY). We specifically provide an overview of population structure, we examine linkage disequilibrium in the association study panel, and we identify DArT markers associated with the traits mentioned above.

Materials and methods

Phenotypic evaluation

A total of 155 inbred lines (Online Resource 1) derived from West and Central African landraces (open-pollinated varieties) were evaluated in multiple field trials in four WA countries, namely Burkina Faso (Gampela; 12°25′51″N; 1°22′18″W), Niger (Sadoré; 17°36′28.04″N; 8°4′53.99″W), Mali (Koporo; 14°3′49.9″N; 3°4′31″W) and Senegal (Bambey; 14°42′2.66″N, 16°27′32.8″W) in the rainy seasons (RS) of 2011 and 2012 under two P-level treatments: high P (HP; with P fertilization) and low P (LP; without P fertilization). Since pearl millet is naturally outcrossing, the open-pollinated varieties experienced a high degree of inbreeding depression, and as a result, the inbred lines were developed using initial selfing for three generations, followed by two generations of sibbing and then a last generation of selfing. The trials were sown side by side in α-lattices with three replications within each P-fertilization level. Individual plot size was 3.6 m^2, which comprised of a single-row plot of 7 hills with 0.8 m intra- and 0.75 m inter-row spacing. Trials were rainfed, with total locational rainfall ranging from 466 to 950 mm across the location × year combinations. Initial soil testing was done by sampling the top 20 cm and analyzing for pH, total nitrogen, organic carbon, Bray1-P and exchangeable potassium; pH ranged from 4.6 to 6.6, total nitrogen ranged from 83 to 370 mg N kg^{-1} soil, organic carbon ranged from 0.11 to 0.41 %, Bray1-P ranged from 3.0 to 7.7 mg P kg^{-1} soil, and exchangeable potassium ranged from 0.11 to 0.47 $cmol^{+}$ kg^{-1} soil. The soil sampling was carried out

by taking five representative samples per replication in each P-level, which were then mixed and soil analysis was done for each replication for each P-level. Growing conditions for these field trials are described in detail in Gemenet et al. (2014). In RS 2011, the HP treatment received a basal 20 kg P ha^{-1} and 18 kg N ha^{-1} [as 100 kg ha^{-1} diammonium phosphate (DAP)], whereas the LP treatment was only supplied with 18 kg N ha^{-1} (as 39 kg ha^{-1} urea). Two top dressings, of 16 kg N ha^{-1} (as 35 kg ha^{-1} urea) each, were supplied to each plot at 30 and 45 days after sowing. Drought that occurred early in the season within the region in RS 2011 caused delay of fertilizer applications to the trials by up to 2 weeks after sowing to avoid burning the seedlings. Based on the results from RS 2011, and concerns over a masked P effect, in RS 2012 the HP treatment received a basal 40 kg P ha^{-1} [as 100 kg ha^{-1} DAP + 255.56 kg ha^{-1} single super phosphate (SSP)] and 18 kg N ha^{-1} (as 39 kg ha^{-1} urea) followed by four topdressings with 11.4 kg N ha^{-1} (as 25 kg ha^{-1} urea) at 3, 5, 7 and 9 weeks after sowing. Data collected include time to 50 % flowering (FLO; days from sowing to full female stigma emergence on 50 % of the main stem panicles per plot) and grain yield (GY; g m^{-2}). In addition, P concentration in stover (PCS; mg g^{-1}) and P concentration in grain (PCG; mg g^{-1}) were measured using an inductively coupled plasma emission spectrometer (ICP-OES) according to VDLUFA (2011) using air-dried samples obtained from the Sadoré 2011 HP and LP trials. P uptake (PBM, mg m^{-2}) was then conservatively calculated as the sum of total P in grain (PCG * GY; mg m^{-2}) and total P in stover (PCS * SWT; mg m^{-2}, where SWT = stover weight, obtained by air drying the stover per plot to constant weight). P-utilization efficiency (PUE) was calculated as the ratio of grain yield and total P uptake (GY/PBM; g mg^{-1} P). Field evaluation of the inbred lines for GY is discussed in detail in Gemenet et al. (2014), whereas P uptake and PUE of the inbred lines is discussed in detail in Gemenet et al. (2015a).

Genotypic evaluation

DNA was extracted from leaves of a single 3-week-old plant per inbred line using the QIAGEN DNeasy mini-plant kit. DArT marker genotyping was done by the Genomics Service Laboratory of the M.S. Swaminathan Center of Excellence in Genomics, which is located on the Patancheru campus of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), in Hyderabad, India. DArT analysis involves reducing the complexity of a DNA sample to obtain a 'representation' mainly based on restriction, adapter ligation and then amplification. The reasoning behind this is that a representation contains two types of fragments: common fragments for a species and variable fragments present in some but not in other individuals of a species. The second category is considered as DArT markers, and once a library of a species is formed (DArT array), the presence/absence of the variable markers can be scored in any representation hybridized to the DArT array. A DArT array for pearl millet was developed at ICRISAT, India, based on diverse genotypes representing the diversity of pearl millet accessions held at the GenBank using the Pst1-based complexity reduction method by Wenzl et al. (2004). The complete method description of the pearl millet DArT array development is given in Supriya et al. (2011). For analysis of our inbred lines panel, genomic representations were generated for each of our inbred lines as described by Supriya et al. (2011). The representations were then hybridized to the pearl millet DArT array, and polymorphic markers were scored '1' for presence and '0' for absence using DArTsoft as described in Supriya et al. (2011). The DArTsoft generated '1' and '0' scores were provided for a total of 407 DArT markers that were 100 % reproducible with the DNA samples provided, with call rates ranging from 75.0 to 98.2 % and polymorphic information content (PIC) values ranging from 0.10 to 0.50 with an average of 0.35. The number of markers was reduced to 285 by restricting the minimum call rate to $\geq$90 % per marker. The list of markers used in the present study is provided in Online Resource 2, and more details concerning the clones from which the present markers were developed can be requested from the Genomics Service Laboratory of the M.S. Swaminathan Center of Excellence in Genomics. The 285 markers were used in further analysis with 151 genotypes (four genotypes were excluded due to too much missing genotypic data).

Data analysis

Phenotypic data analysis

Analysis of the phenotypic data was based on REML mixed models in GENSTAT 17th edition. Data from Koporo 2012 and Bambey 2012 were left out from

analysis due to too many missing data points as a result of too much rainfall directly after sowing with concomitant soil erosion/compaction that affected trial establishment. In order to avoid double shrinkage associated with the use of best linear unbiased predictors (BLUPs) in association analysis (Piepho et al. 2012), best linear unbiased estimators (BLUEs) were calculated for each trait considering genotypes as fixed and both replications and blocks nested within replications as random effects in single-environment analyses (environment = P-level × location × year combination). For combined analysis within one P-level, across locations and/or locations and years, genotypes were considered fixed, while locations, years and all interactions as well as replications nested within locations and blocks nested within replications, were considered random. For combined analysis across P-levels, the genotypes and P-level treatments were considered fixed, whereas environments (location × year combination) and all interactions as well as replications nested within environments and blocks nested within replications, were considered random. In our data set, PUE had previously been shown to be highly positively correlated with grain harvest index (HI), while FLO was negatively correlated with GY (Gemenet et al. 2015a). In the present study, HI was therefore used as a fixed regression factor in the fixed model for PUE, while FLO was also used as a fixed regression factor in the fixed model for GY (Sabadin et al. 2012). Variance components were then estimated by fitting the above models with genotypes as random. Repeatability estimates (w^2) for single-environment analysis as well as broad-sense heritability (h^2) for combined analysis were calculated as:

$$w^2 = \sigma_g^2 \Big/ \left[\sigma_g^2 + (\mathrm{VD}/2)\right]$$

where VD is the average variance of a difference between means of genotypes and σ_g^2 is the genetic variance component (Piepho and Möhring 2007).

Genetic correlations between HP and LP were calculated following Cooper et al. (1996) as:

$$r_{g(\mathrm{HP,LP})} = r_{p(\mathrm{HP,LP})} \Big/ \left(w_{\mathrm{HP}}^2 \times w_{\mathrm{LP}}^2\right)^{1/2}$$

where $r_{p(\mathrm{HP,LP})}$ is the correlation between genotypic means under HP and LP conditions and w_{HP}^2 and w_{LP}^2 are the repeatability estimates (= broad-sense heritability h^2 for combined analysis) under HP and LP conditions, respectively.

Relationships between the environments were visualized using a genotype and genotype-by-environment (GGE) biplot (Yan and Kang 2002).

Inference of population structure

Population structure was examined based on the 285 DArT markers (scored as presence/absence) using a model-based approach implemented in STRUCTURE software (Pritchard et al. 2000), using the admixture model with correlated allele frequencies and without prior information. The membership of each genotype was run for the range of genetic clusters (K) from $K = 1$ to $K = 10$ with each run consisting of 100,000 steps of burn-in followed by 100,000 replications using Monte Carlo Markov chains (MCMC) with five repetitions for each K. The optimal levels of likelihood $L(K)$ and the *ad hoc* criterion ΔK were determined from the STRUCTURE files using STRUCTURE HARVESTER (Earl and vonHoldt 2012). To avoid stochastic effects of replicated STRUCTURE runs, the results were collated using the program CLUMPP (Jakobsson and Rosenberg 2007). Furthermore, a pair-wise genetic dissimilarity matrix was calculated based on the Jaccard index implemented in DARwin 5.0.158 software (Perrier and Jacquemoud-Collet 2006). Based on the dissimilarity matrix, genotypes were assigned into clusters using the unweighted neighbor-joining method with 1000 bootstraps. A Q-matrix at $K = 3$ was used in linkage disequilibrium analysis and as a covariate matrix in association analysis.

Linkage disequilibrium analysis

As we did not have information on the genetic position of most of the markers used in this study, we did not examine patterns of LD in the entire set and within-population structure subgroups but rather restricted LD analysis to markers significantly associated with traits to determine their independence. Linkage disequilibrium between marker pairs was analyzed using TASSEL 4.2.1 (Bradbury et al. 2007) and was quantified mainly based on squared correlation coefficients (r^2) between loci. Loci were considered to be in significant LD when p value <0.01.

Model selection

We tested both general linear models (GLM) and mixed linear models (MLM) to calculate p values for associating each marker with the trait of interest, along with accounting for population structure to avoid spurious associations. The population structure (Q-matrix) from STRUCTURE at $K = 3$ was used as a covariate to correct for population structure. The kinship matrix (K-matrix) used in MLM analysis was calculated with the 285 DArT markers using TASSEL 4.2.1 (Bradbury et al. 2007). The Q-matrix, K-matrix and the phenotypic data were fitted using restricted maximum likelihood (REML) in the SAS version 9.4 mixed procedure (SAS institute 2015). In the K-models, we assumed that all genotypes are correlated according to the K-matrix and therefore this K-matrix was represented as a linear variance–covariance matrix in the model. In the models without K (Q-models), genotypes were considered independent. The denominator degrees of freedom were synthesized based on the method of Kenward and Roger (1997). Since the number of covariates required to correct for population structure varies for each trait, we tested models with $Q = 1$, and $Q = 2$ (as when using the $K = 3$ Q-matrix, the sum of all three Q values equals 100 % and creates linear dependency in the analysis that can be avoided by excluding one of the Q values; Ramdoss et al. 2011). The simultaneous significance of both Qs in the model was tested based on an F test using the SAS contrasts statement and which Q was more important for each trait was compared using solutionF statement. Corrected Akaike's information criterion (AICc) computed using REML was used to compare between the Q- and the $K + Q$-models. We therefore in the end had tested the following models: (1) GLM without any correction for population structure (naive model), (2) GLM with Q-matrix as correction for population structure (Q-model), (3) MLM with kinship matrix as correction for relatedness (K-model), (4) MLM with Q- and K-matrices as correction for population structure and relatedness ($Q + K$-model, using the levels of Q listed above) for each of the 38 traits presented herein. The SAS code used in model selection is given as Online Resource 3.

Association analysis and criteria for determining significant associations

A two-step association mapping (Stich et al. 2008) was applied using BLUEs from single field trials as well as from combined analyses. Association analysis was carried out based on the best identified model from the model selection step above in TASSEL 4.2.1 (Bradbury et al. 2007). The Bonferroni correction for false positives at 5 % (0.05/number of markers; maximum p value $= 1.8 \times 10^{-4}$ in this case) was found to be too stringent for most of the traits with this number of markers. Since population structure effects and most of the false positives had been inherently controlled by the selected associated model, a less stringent approach proposed by Chan et al. (2010) working on *Arabidopsis* and also applied by Pasam et al. (2012) in spring barley was considered for determining the threshold level for significant marker–trait associations. They had suggested that the bottom 0.1 percentile distribution of the p values is considered as significant, which in our analysis resulted in threshold levels of $-\log(p \text{ values}) \geq 2$ for individual traits. Based on this and the studies by Hao et al. (2012) and Baskaran et al. (2014), we passed significant association at $-\log p > 2.00, p < 0.01$ threshold as a first step. This resulted in several significant associations for each trait. Since adjusting family-wise error is important in multiple testing, we additionally analyzed the p values based on the false discovery rates (FDR) model proposed by Benjamini and Hochberg (1995) at $p < 0.1$. We declared significant marker–trait association for markers which passed the FDR test and/or retained only the first marker with the lowest p value for traits where none of the selected p values passed the FDR test.

Results

Genetic variation and performance under low-phosphorus conditions

All observed and calculated traits showed large and significant genotypic variation (Table 1). Repeatability estimates (w^2) ranged from 0.45 to 0.89 under LP and 0.56–0.91 under HP (Table 1). Repeatability

Table 1 Best linear unbiased estimators (BLUEs; μ) means, genetic variance components (σ_g^2) and repeatability estimates (w^2) of traits measured under low-phosphorus (LP) and high-phosphorus (HP) conditions as well as the genetic correlations (r_g) between traits measured on pearl millet inbred lines under LP and HP in six environments (site–year combinations) in West Africa

Year	Location	Trait	LP			HP			HP,LP
			μ	σ_g^2	w^2	μ	σ_g^2	w^2	r_g
2011	Sadore	PCS	0.7	0.05***	0.74	0.8	0.05***	0.64	0.92
		PCG	2.3	0.04***	0.62	2.6	0.07***	0.72	0.95
		PBM	220.3	487***	0.60	254.4	644***	0.77	0.94
		PUE	0.3	[a]9.97***	0.56	0.2	[a]8.53***	0.62	0.99
		FLO	65.6	20.5***	0.89	61.3	15.7***	0.88	0.98
		GY	62.6	528***	0.57	67.0	783***	0.71	0.85
	Koporo	FLO	72.6	22.4***	0.84	71.1	20.0***	0.81	0.98
		GY	79.5	939***	0.59	87.1	1184***	0.60	0.97
	Gampela	FLO	66.5	17.1***	0.77	64.5	11.5***	0.84	0.90
		GY	65.1	609***	0.52	91.8	1062***	0.66	0.53
	Bambey	FLO	64.5	34.2***	0.73	61.4	33.8***	0.79	0.91
		GY	60.7	1178***	0.71	69.9	1911***	0.72	0.99
2012	Sadore	FLO	68.2	42.7***	0.78	61.5	19.3***	0.84	0.73
		GY	23.2	173.3***	0.45	36.2	330.1***	0.56	0.80
	Gampela	FLO	66.6	19.8***	0.74	63.2	15.2***	0.91	0.84
		GY	53.6	298***	0.57	68.2	747***	0.58	0.77

PCS = P concentration in stover (mg g^{-1}), PCG = P concentration in grain (mg g^{-1}), PBM = P in total biomass (P uptake; mg m^{-2}), PUE = P-utilization efficiency corrected for harvest index (g mg^{-1} P), FLO = days to flowering (days), GY = grain yield (g m^{-2})

*** Significant at $p < 0.001$

[a] Variance component multiplied by 10,000 for easy readability

estimates were in most cases reduced under LP cf. HP. Except for FLO and PUE, which had higher means under LP, most traits had reduced means under LP although the differences between the means were not very large between the HP and LP treatments in a given site × year pair of environments (Table 1). Genetic correlations between performance under HP and under LP were consistently high, ranging from 0.53 to 0.99 (Table 1). For combined analysis across locations within one P-level, broad-sense heritability (h^2) was 0.67 under LP and 0.76 under HP for GY and 0.93 under LP and 0.94 under HP for FLO (Online Resource 4). For combined analysis across P-levels, broad-sense heritability (h^2) was 0.81 and 0.97 for GY and FLO, respectively (Online resource 4). Variance components for different sources of variance in the combined analyses for GY are provided in detail in Gemenet et al. (2014). The ratio of the genotypic variance component to that of genotype-by-P-level interaction (G:G × P) was 1:0.05, whereas the genotypic variance component ratio to that of genotype-by-environment (G:G × E; environment = location × year combination) was 1:0.52 (Gemenet et al. 2014). The GGE biplot shows that most environments were not very differentiated in the current study as all environments appear in two very close sectors except Bambey 2011, which was clearly separated from the other environments (Online Resource 5). No mega-environments were observed for P-levels in the GGE biplot (Online Resource 5).

Population structure

STRUCTURE results indicated a maximum ΔK at $K = 3$ (Online Resource 6) indicating three subgroups among the 155 genotypes. Assignment at >0.8 probability based on the collated $K = 3$ clustering could apportion 31.8 % of the inbred lines to subgroups, >0.7 could apportion 47.3 % inbred lines to subgroups, and >0.6 could assign 68.9 % inbred lines to

subgroups. These three subgroups were also observed from the unweighted neighbor-joining tree as three major groups each with its own subgroups (Fig. 1). These three major subgroups appear to have been grouped mainly based on flowering time because Q1 was negatively correlated with time to 50 % flowering (FLO; $r = 0.44$), and Q2 was positively correlated with FLO ($r = 0.40$), while Q3 did not show significant correlation with FLO (data not shown).

Associations between phenotypes and genotypes

Model selection

Several models were compared to assess their usefulness in accounting for population structure and their ability to reduce the inflation of false positive associations (Type I error). The AICc values for the $K + Q$- and the Q-models, together with the selected model and the number of Qs required to control for population structure, if any, are shown in Online Resource 7. In most cases, controlling for population structure was sufficient for most flowering time traits while controlling for relatedness rather than population structure was more appropriate for grain yield traits. The model comparison step is also graphically presented for combined GY across environments (location × year combination) and P-levels (GY_HLP_Com), which is one of the 38 traits analyzed in the current study in Online Resource 8, where ranked p values for each model were cumulatively plotted. It can be observed that the naive model performed similarly to both of the Q-models. The K-model and the two $Q + K$-models also performed

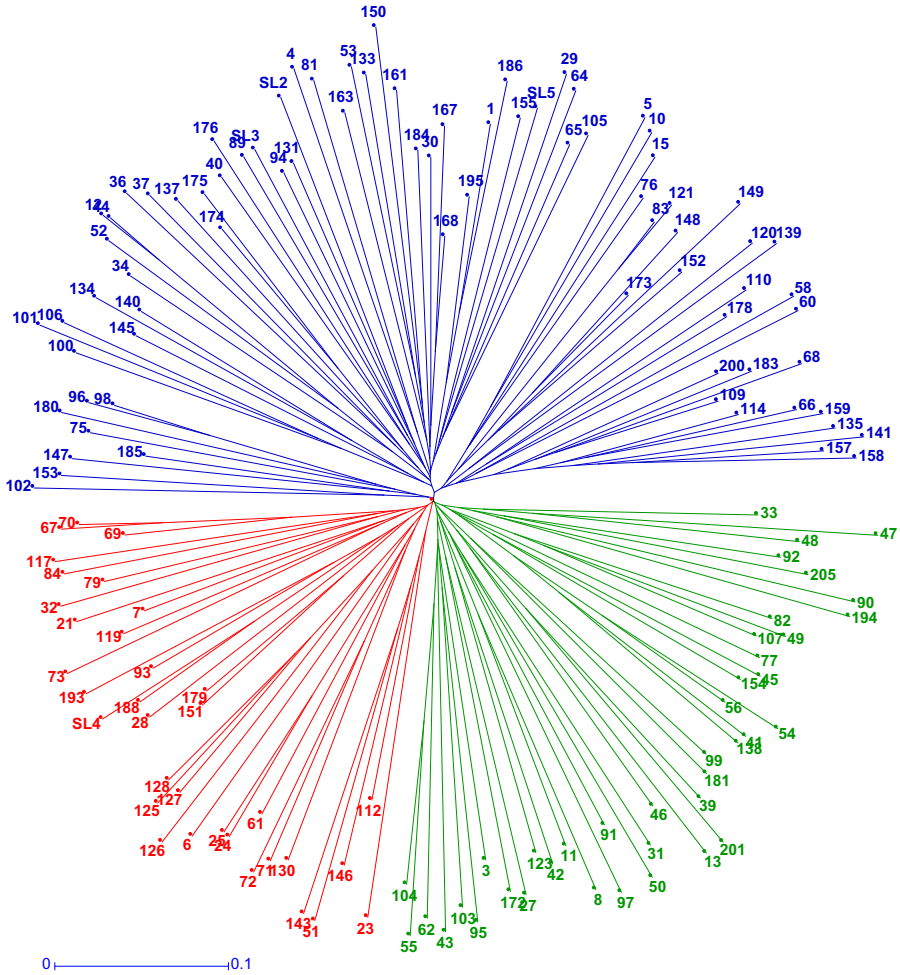

Fig. 1 Unweighted neighbor-joining tree based on dissimilarities in 151 pearl millet inbred lines from WA using 285 DArT markers. The *colors* represent the three main subgroups. Scale (0–0.1) indicates genetic distance. The *numbers* correspond to the inbred line numbers which are indicated by the last three digits of the inbred line names as presented in Online Resource 1. (Color figure online)

similarly (Online Resource 8), and being a grain yield trait, it can be seen that controlling for relatedness was more desirable. There was a low level of relatedness in the inbred lines as can be seen in the kinship heat map presented as Online Resource 9.

Marker–trait associations for phosphorus-efficiency-related traits

Nine markers were found to be significantly associated with the four P-efficiency-related traits (Table 2). The markers individually explained about 7–16 % of the observed variance in the respective traits (Table 2). One marker each was significantly associated with reduction in PCG under HP and LP. Two markers were significantly associated with increased PCS under HP, while one marker was associated with reduced PCS under LP. Three markers, one of which was associated with increased PBM, while two were associated with reduction in PBM, were found significant under HP, while one marker associated with reduced PBM was significant under LP. One marker each under HP and LP was significantly associated with reduced PUE. Marker *PgPb7101* was significantly associated with three of the four P-efficiency-related traits under HP, being significantly associated with increased PCS, increased PBM and reduced PUE (Table 2).

Table 2 Markers significantly associated with phosphorus (P) efficiency-related traits: P concentration in grain (PCG; mg g^{-1}), P concentration in stover (PCS; mg g^{-1}), P-uptake efficiency (P in total biomass; PBM; mg m^{-2}) and P-utilization efficiency (PUE; g mg^{-1} P) measured in pearl millet inbred lines under low-phosphorus (LP) and high-phosphorus (HP) conditions at Sadore (Niger) in 2011, their *p* values, the percentage of variance explained by the association and the estimated marker effects

Trait	Marker	*p* value	% variance	Effect[a]
PCG _HP	PgPb12598	6.7×10^{-3}	6.9	−0.13
PCG_LP	PgPb12839	3.3×10^{-3}	8.6	−0.20
PCS_HP	PgPb7101	8.5×10^{-6}	15.9	0.74
	PgPb8535	9.6×10^{-4}	9.1	0.21
PCS_LP	PgPb8177	7.7×10^{-4}	8.4	−0.25
PBM_HP	PgPb7101	4.2×10^{-5}	14.4	68.0
	PgPb11170	4.6×10^{-5}	14.3	−12.1
	PgPb8935	3.7×10^{-4}	11.1	−20.4
PBM_LP	PgPb7983	6.8×10^{-4}	10.2	34.0
PUE_HP	PgPb7101	1.0×10^{-4}	11.2	−0.01
PUE_LP	PgPb13376	6.4×10^{-3}	6.9	−0.03

[a] Marker effects refer to presence of the marker

Marker–trait associations for days to flowering

A total of nine markers were found to be significantly associated with FLO (Table 3), each individually explaining about 5.5–9.9 % of the observed variation for flowering time. Four of these markers were found significantly associated with FLO in only one environment, while the remaining five were found significantly associated with FLO in more than one environment. The markers with significant association with FLO in more than one environment are highlighted in bold in Table 3. Marker *PgPb11603* appeared to be the most stable as it was found significant in Sadore in both 2011 and 2012 and was also the marker significantly associated with combined effects for FLO under both HP and LP. Most markers did not show specificity for either HP or LP (Table 3).

Marker–trait associations with grain yield

A total of 13 markers individually explaining about 7.2–15.6 % of the observed variation were found to be significantly associated with GY (Table 4). Ten of these markers were significantly associated with GY in only a single environment, while the remaining three: *PgPb12954, PgPb10876* and *PgPb11459* were found to be significantly associated with GY in more than one environment and/or combined effects. Marker *PgPb12954* had the most stable associations with increased GY of between 10.0 and 21.0 g m^{-2} (Table 4).

Colocation of markers for different traits

Two markers were found to be significantly associated with more than one trait. Marker *PgPb6780*, which was significantly associated with reduced FLO in Gampela and Sadore in 2011 (Table 3), was also associated with increased GY in Gampela 2011 (Table 4), whereas marker *PgPb7101*, which was found significantly associated with increased PCS, increased PBM and reduced PUE, was also associated with increased GY in Gampela 2011.

Table 3 Markers significantly associated with days to flowering (FLO; days) measured on pearl millet inbred lines under high-phosphorus (HP) and low-phosphorus (LP) conditions (P-level) in six environments (site–year combinations) in West Africa, their p values, the percentage of variance explained by the associations and the estimated marker effects

Year	Location	P-level	Marker	p value	% variance	Effect[a]
2011	Bambey	HP	**PgPb5985**	2.1×10^{-3}	7.0	−1.5
		LP	**PgPb6723**	4.9×10^{-4}	7.7	1.9
	Gampela	HP	**PgPb6780**	6.7×10^{-4}	9.9	−4.5
		LP	PgPb6798	1.5×10^{-3}	8.4	−1.4
	Koporo	HP	**PgPb5985**	2.5×10^{-4}	7.8	−1.7
			PgPb6723	9.7×10^{-4}	6.6	1.6
		LP	**PgPb5985**	2.3×10^{-4}	8.2	−2.1
	Sadore	HP	**PgPb6780**	1.5×10^{-3}	6.3	−3.8
			PgPb11603	1.8×10^{-3}	6.1	−1.7
		LP	PgPb10709	4.0×10^{-4}	5.5	0.6
2012	Gampela	HP	PgPb12084	3.2×10^{-4}	9.0	−2.1
		LP	**PgPb12472**	2.6×10^{-3}	7.3	−4.3
	Sadore	HP	**PgPb11603**	1.8×10^{-3}	8.6	−2.4
			PgPb12306	1.9×10^{-4}	8.4	−3.6
		LP	**PgPb11603**	1.9×10^{-3}	7.7	−1.0
Combined	Across	HP	**PgPb11603**	7.8×10^{-4}	6.9	−2.0
		LP	**PgPb11603**	2.0×10^{-3}	6.1	−1.6
		HP and LP	**PgPb11603**	8.9×10^{-4}	6.8	−1.8
			PgPb12472	2.3×10^{-3}	5.9	−2.9

[a] Marker effects refer to the presence of the marker

Bold indicates the probability of marker-trait association

Linkage disequilibrium between markers

A few of the markers reported to have significant associations with various traits related to performance under contrasting P-levels as reported were found to be in significant LD $p < 0.01$. Marker *PgPb10709* found to be associated with increased FLO by about 0.6 days was in significant LD with marker *PgPb12472* (Table 3), which was also associated with reduced FLO, and marker *PgPb11459*, which was associated with reduced GY (Table 4). Marker PgPb11459 was also in significant LD with marker *PgPb10217*, which was also associated with reduced GY (Table 4). Marker *PgPb7461* significantly associated with reduced GY in Bambey 2011 was in significant LD with marker *PgPb6628* associated with increased GY in Sadore 2011 and marker *PgPb7101* (Table 4) significantly associated with increased GY in Gampela 2011. Markers *PgPb7983* and *PgPb1170* (Table 2) both associated with PBM were also in significant LD.

Discussion

Low level of differentiation between P-levels in field evaluation

Low-P conditions have been reported to lead to reduced GY and delayed FLO in sorghum, (Leiser et al. 2012), maize (Parentoni et al. 2010) and common bean (Beebe et al. 2007). Although we observed delayed FLO and reduced GY across all environments under LP conditions, as well reduced means for PCG, PCS and PBM under LP; the observed differences between the P-levels were not very large, and the genetic correlation values between HP and LP were high (indicative of pearl millet's relatively good tolerance to the low-P conditions used in these trials). This can also be observed in the GGE plot where the angle between HP and LP was always small with no mega-environments observed for P-levels in all environments (site × year combinations) and implies that both of our P-levels rank the 155 pearl millet inbred

Table 4 Markers significantly associated with grain yield (GY; g m^{-2}) measured on pearl millet inbred lines under high-phosphorus (HP) and low-phosphorus (LP) conditions (P-level) across six environments (site-year combinations) in West Africa, their *p* values, the percentage of variance explained by the associations and the estimated marker effects

Year	Location	P-level	Marker	*p* value	% variance	Effect[a]
2011	Bambey	HP	PgPb7461	7.5×10^{-4}	8.8	−34.8
		LP	**PgPb12954**	1.6×10^{-3}	8.8	21.0
	Gampela	HP	PgPb6780	2.8×10^{-3}	8.2	16.5
		LP	PgPb7101	4.0×10^{-3}	7.2	17.9
	Koporo	HP	PgPb10674	4.8×10^{-5}	14.2	47.1
			PgPb12954	1.1×10^{-3}	9.5	16.0
		LP	**PgPb10876**	8.2×10^{-4}	10.0	20.4
			PgPb12954	1.1×10^{-3}	9.5	15.8
	Sadore	HP	PgPb6628	2.9×10^{-3}	8.1	20.4
		LP	PgPb11235	5.7×10^{-4}	10.5	12.2
2012	Gampela	HP	**PgPb12954**	4.9×10^{-4}	10.7	10.3
			PgPb11056	4.9×10^{-4}	10.7	13.3
		LP	PgPb9967	1.9×10^{-3}	8.8	0.6
	Sadore	HP	PgPb10217	6.1×10^{-4}	9.6	−4.6
		LP	**PgPb10876**	2.5×10^{-5}	15.6	26.1
			PgPb10468	2.1×10^{-4}	12.3	31.1
Combined	Across	HP	**PgPb12954**	2.3×10^{-4}	11.8	10.3
			PgPb11459	1.0×10^{-3}	9.6	−21.7
		LP	**PgPb12954**	1.2×10^{-4}	12.8	11.0
			PgPb10876	8.8×10^{-4}	9.8	17.6
		HP and LP	**PgPb12954**	1.1×10^{-4}	12.9	10.0
			PgPb10876	9.5×10^{-4}	9.7	21.0
			PgPb11459	1.8×10^{-3}	8.8	−18.4

[a] Marker effects refer to the presence of the marker

Bold indicates the probability of marker-trait association

genotypes in a similar manner. In Gemenet et al. (2014), we reported small relative yield reductions across locations with a mean of 23.5 % in the combined analysis. This occurred despite our adding a substantial amount of P to the HP treatment and implies that the P effect was masked (at least partially) in these experiments. According to Valluru et al. (2010), early season P-deficiency results in early irreversible growth restriction in pearl millet. Year 2011 was a major drought year within the study region (Haesler 2012), and drought occurring early in the rainy season led to delay of fertilizer application by as much as >2 weeks (to avoid fertilizer-induced burning of seedlings due to inadequate soil moisture). This could largely explain the lack of a strong P effect in the evaluations conducted in 2011 (and across the 2011 and 2012 rainy seasons). However, this was not the only reason for the small observed effect of P-levels in this study as in 2012 there was enough rainfall at sowing time, the fertilizer treatments were applied at the time of sowing, and the amount of P was doubled, but still no strong P effect was observed. This implies that besides late P-application in 2011, other soil-related factors were in play. It has long been pointed out that in environments that are less favorable for agricultural production such as those of the West African Sahel, moisture and soil toxicity constraints interact very strongly with soil nutrient availability to the extent that it even becomes difficult to obtain economic responses to individual fertilizers and/or other soil amendments (Brück et al. 2000; Payne et al. 1995; Schaffert et al. 2000; Zaongo et al. 1994; Subbarao et al. 2000; Hash et al. 2002) except compost or farmyard manure. Aluminum (Al) toxicity and P

fixation due to mineral compositions high in iron (Fe) and Al oxides are common in tropical acid soil savannas including the WA Sahel (Schaffert et al. 2000; Weir 1972, 1977; Hash et al. 2002). Despite these limitations, we observed high enough repeatability estimates (and broad-sense heritabilities for combined analyses) with substantial genetic variation for all directly observed and calculated traits, and these data could therefore be used in further analysis.

Subtle population structure and familial relatedness in the study panel

Populations used in association studies are classified into five groups as (1) ideal samples with subtle population structure and little-if-any familial relatedness, (2) multi-family samples, (3) samples with population structure, (4) samples with both population structure and familial relationships and (5) samples with severe population structure and familial relationships (Yu and Buckler 2006; Yu et al. 2006; Zhu et al. 2008). In the current study, we observed three population structure subgroups, mainly based on FLO. This is expected because flowering time has been shown to be a major adaptive trait in crop plants such as maize and pearl millet (Camus-Kulandaivelu et al. 2006; Stich et al. 2010). Studies on pearl millet diversity in WA have not reported any substantial differentiation of pearl millet based on geographic distance or agroecological zones (Tostain et al. 1987; Oumar et al. 2008; Stich et al. 2010). Genetic diversity based on simple sequence repeat markers in the current West African pearl millet inbred germplasm association panel (WA-PMiGAP) is reported in detail by Stich et al. (2010). According to Pucher et al. 2015, flowering time is differentiated in pearl millet based on latitude with varieties toward the north being more earlier flowering compared with varieties preferred for the south. This could also be the reason why in the present study controlling for population structure and not so much of kinship was important for FLO. The presence of strong population structure may result in Type I error if not accounted for (Zhu et al. 2008). Whereas several statistical models have been proposed to account for population structure in association analysis (Yu et al. 2006; Zhao et al. 2007), there is need for balancing the rates of false positives and false negatives (Pasam et al. 2012), so several of these models were tested in the present study. It is evident from the present study that the observed population structure within WA-PMiGAP was not very strong as the Q-model and naive model performed almost similarly in accounting for population structure (see Online Resource 8). Familial relatedness was also low as can be observed from the kinship matrix provided as Online Resource 9. Baskaran et al. (2009) similarly observed few differences between marker–trait associations detected using a naive model and one including population structure, in a pearl millet study involving five sets of full-sib progenies generated from populations along a chain of five random-mating populations starting with a new base population derived from a population cross made at ICRISAT, Patancheru, and an improved open-pollinated variety derived from that population which was developed, tested and released for cultivation in Tamil Nadu state in southern India. In that case, the random-mated base population and its four sequentially derived populations were each based on a recombination of at least 50 full-sib progenies and hence expected to be in nearly complete linkage disequilibrium. As subsequently demonstrated (Baskaran et al. 2014), this means that use of codominant markers with highly heterozygous full-sib progeny sets developed from a truly random-mating population can be very effective for detection of marker–trait associations—even without taking into account population structure, as population structure accounts for less than 1 % of the observed genetic variance in such cases. This in turn means that marker-assisted population improvement (MAPI) using full-sib progenies can be expected to be very efficient—and directly applicable for improvement of highly heterozygous open-pollinated varieties of seed-propagated crops or clonally propagated crops. Applied marker-assisted selection is not just for those self-pollinated crop species (or cross-pollinated species where inbreeding is practical), where marker-assisted pedigree selection is practical. It also has tremendous potential for use in applied improvement of allogamous species (crops, livestock, etc.).

Significant marker–trait associations

Several statistically significant putative marker–trait associations were identified for P-related traits, FLO and GY. Most markers involved in these putative marker–trait associations were not in significant LD with each other implying that they segregate independently.

Low-P tolerance-related traits

Here, we present the first report of association mapping for low-P tolerance-related traits in pearl millet. Significant marker–trait associations were observed for PCG, PCS, PBM and PUE. QTLs associated with low-P tolerance traits have been reported previously in several crops such as rice (Wissuwa et al. 2002), wheat (Su et al. 2009), common bean (Beebe et al. 2006), soybean (Zhang et al. 2009; King et al. 2013), barley (Gahoonia and Nielsen 2004), maize (Chen et al. 2009, 2011) and sorghum (Hufnagel et al. 2014). Most of these studies examined P efficiency in early plant growth stages, for which applied utility as a secondary trait in plant breeding is dependent upon both secondary trait heritability and its correlation with GY (Gemenet et al. 2015a). Few previous studies have examined these traits at maturity under field conditions. Mendes et al. (2014) identified six QTLs associated with P-uptake efficiency and five QTLs associated with P-utilization efficiency in maize under field conditions. The current findings therefore offer new insights for breeding programs aiming to improve P efficiency in pearl millet. To be useful in marker-assisted selection programs targeting low-P environments, the putative marker–trait associations identified in this study need to be validated either (1) across environments and in different genetic backgrounds or (2) more quickly and less expensively (at least for pearl millet) by direct selection for and against specific marker alleles within the WA-PMiGAP, recombination of replicated selected subsets of this inbred panel (that is, groups of inbreds that either have or do not have the specific presence/absence marker of interest for a given target trait), and replicated field testing of the replicated recombined pairs of subpopulations under HP and LP conditions. Such validation is required as the P-efficiency-related traits in this study were measured in only one location (but several environments), and some evidence of the desired response to selection is necessary before more routine use of these DArT markers (or others found to be genetically linked to them) can be recommended.

Days to flowering and grain yield

Our strategy to detect marker–trait associations for FLO and GY measured in different locations and years attempted to identify every possible marker–trait association in each testing environment, as well as capturing those markers with effects detected in multiple environments. Most markers significantly associated with these traits did not show specificity for either HP or LP. This is probably because of the lack of a strong response to P fertilization (i.e., P effect) in our field trials (Gemenet et al. 2014), which implies that plant growth in both P-levels may have been limited by some other factor (perhaps the P-fixation capacity of the Fe- and Al-rich soils on which the field experiments were conducted).

We found several significant associations for FLO with some markers exhibiting stable associations with this trait across environments, while others detected associations that were specific for single environments. Being a major adaptive trait in pearl millet, genomic regions contributing to variations in FLO have been shown to be present in all seven linkage groups of pearl millet (Hash et al. 1995, 2003; Yadav et al. 2002, 2003). Clotault et al. (2012) and Lakis et al. (2012) suggested that the wide variation in FLO in pearl millet is likely to be under the influence of several genes. A majority of the presence/absence-scored DArT marker involved in associations with FLO that were identified in the current study were associated with early flowering—the only explanation for this is chance, as a representative random sample of such marker–trait associations would be expected to have about half showing negative additive effects and the other half showing positive additive effects. Saidou et al. (2009, 2014) showed that the region around the PHYTOCHROME C (*PHYC*) gene is responsible for FLO variation in pearl millet and identified an early-flowering allele within the *PHYC* region. Similarly, two independently segregating, recessively inherited genes, e_1 and e_2, were previously shown to confer photoperiod-insensitive early flowering in pearl millet (Anand Kumar and Andrews 1993), and many other relatively early-maturing pearl millets exhibit dominant or partially dominant early flowering that is very useful in breeding early-maturing hybrids (e.g., A/B-pairs 834A/B, 842A/B, 843A/B, 863A/B and ICMA/B 88004, all of which appear to have an early-maturing, bold-seeded, agronomically elite *Iniari* landrace-based parentage (Andrews and Kumar 1996; Stegmeier et al. 1998a, b; Rai et al. 1995, 2008). Due to the current lack of information concerning map positions of most of the markers identified to be significantly associated with FLO in the current study,

it is not possible yet to compare them with previously reported QTLs and/or genes associated with flowering time in pearl millet.

Future sequencing of the DArT clones upon which these markers are based, combined with pending release of the aligned pearl millet genome sequence, will soon permit us to overcome this minor academic inconvenience.

Several DArT markers showed significant associations with GY, having effects in single environments and/or in multiple environments. This is the first study to examine GY performance under LP conditions in pearl millet in replicated field trials. The main reason for capturing both specific- and multiple-effect marker–trait associations in the current study is that both FLO and GY are complex traits exhibiting strong G × E interaction (Kraakman et al. 2004), with specific flowering alleles in specific genomic regions being more favorable in some environments and less favorable in other environments (e.g., Yadav et al. 2003); hence, selection for appropriate flowering time in the target environment itself, or in an artificially manipulated photoperiod-temperature regime that mimics the target environment, is required for conventional breeding approaches to get flowering time 'right.' As flowering time is among the most highly heritable of traits when the physical environment (moisture, temperature, light and nutrient availability) is favorable, this is relatively easily accomplished for favorable crop production environments. However, in more challenging environments, such as those represented by the narrow bands of mean annual precipitation isohyets across inland WA, selection for the desirable photoperiod–temperature response is already a challenge for conventional breeding programs—even before bringing the possible role of nutrient deficiencies or pest-induced delays in effective flowering time into consideration. It is expected that better information about allele-specific associations of numerous marker loci distributed across the entire nuclear genome will soon make it possible to achieve this without the several years of multiple sowing-date observations that are currently required to get the most favorable photoperiod–temperature response of flowering in crop varieties targeted for environments where this is an essential component of local adaptation.

Despite the low differentiation among most of our evaluation environments, as observed from the GGE plot, we identified both stable and environment-specific marker–trait associations with each location showing at least one specific marker–trait association for FLO and/or GY. According to Collins et al. (2008), QTLs can be categorized as being either constitutive (consistently identified across most environments) or adaptive (detected only in specific environmental conditions). We can therefore classify the associations of *PgPb11603* with FLO and *PgPb12954* with GY, which were more stable across environments, as being associated with constitutive QTLs, while most of putative marker–trait associations identified for these two traits in the present study can be considered as being associated with adaptive QTLs. Two propositions are available to explain genetic control of trait stability in multiple environments: (1) where the constitutive gene is itself regulated in direct response to the environment, referred to as the allele-sensitivity model, or (2) where regulatory loci are under the direct influence of the environment and they in turn switch on and off the constitutive genes (Via et al. 1995). More progress in QTL mapping and association analysis for GY in pearl millet has been achieved under terminal drought stress with a major QTL being identified and validated on linkage group 2 (Yadav et al. 2002, 2003, 2004, 2011; Bidinger et al. 2005, 2007; Serraj et al. 2005; Sehgal et al. 2012), for which improved terminal drought tolerance (Kholová et al. 2010a, b) and salinity tolerance (Sharma et al. 2014) appear to be associated with constitutively elevated foliar ABA levels. In contrast, not much information is available on genetic variation in pearl millet GY performance under P-limited conditions. Genomic regions responsible for GY performance under P-limited conditions have been recently reported in sorghum (Leiser et al. 2014; Hufnagel et al. 2014) and maize (Mendes et al. 2014). The findings from the current study therefore will contribute toward bridging the gap between quantitative and molecular methods of studying complex traits like low-P tolerance in West Africa.

Pleiotropy versus close linkage

An interesting contribution of marker–trait association analysis is the possibility of elucidating the genetic basis of associated traits (Tuberosa et al. 2002). The colocation of QTLs for different traits implies the likely presence of pleiotropy or tight linkage between

the QTLs that control the trait (Lebreton et al. 1995; Gemenet et al. 2010; Baskaran et al. 2014). In our case, marker *PgPb7101* had significant association with PCS, PBM and PUE. The negative effect of this marker on PUE together with its positive effect on PBM could just be a reflection of the confounding effects of P uptake on P-utilization efficiency. Rose et al. (2010) suggested that plants with a higher P uptake suffer less from low-P stress and would not show much P-utilization efficiency. This marker was also observed to be associated with increased grain yield in one environment. Several studies have reported colocation of QTLs for P uptake (PBM in this case) and GY in different crops implying the possibility that the two traits are likely to be influenced by same genomic regions. Hufnagel et al. (2014) recently reported that the same genomic region was responsible for P uptake and GY performance under LP conditions in sorghum. As we are dealing with the presence/absence type of markers, whose map positions mostly are unknown, it is not possible to tell with these preliminary findings whether the associations between the two traits in our case are driven by the same molecular polymorphism or by different polymorphisms closely linked (Saidou et al. 2014). Validation of the current putative marker–trait associations is therefore necessary.

Conclusions

We report here the first findings on marker–trait associations for pearl millet under low-P conditions in WA. We observed a subtle population structure and limited familial relatedness, in the germplasm association panel of inbred lines derived from West African landraces and improved open-pollinated varieties (WA-PMiGAP) used in this study. We identified several markers associated with P-efficiency-related traits, time to flowering and/or grain yield. There is a possibility that genomic regions responsible for P-efficiency and GY are colocalized in pearl millet. There is, however, need to further validate the marker–trait associations identified here.

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