No need to breed for enhanced colonization by arbuscular mycorrhizal fungi to improve low-P adaptation of West African sorghums

Willmar L. Leiser · Marcus O. Olatoye · H. Frederick W. Rattunde · Günter Neumann · Eva Weltzien · Bettina I. G. Haussmann

Abstract

Aims Western Africa (WA) sorghums are predominantly cultivated under low plant available phosphorus (P) soil conditions with a diverse population of arbuscular mycorrhizal fungi (AMF) present. This study aims to determine whether sorghum breeding programs should target higher colonization by AMF through understanding the genotypic variation of sorghum for AMF-root colonization (AMF-RC) under different P-fertility conditions at different growth stages and assessing the genetics underlying AMF-RC using genome-wide association study (GWAS).

Method A sorghum diversity panel of 187 WA genotypes was grown in low-P soil in a pot trial for 38 days and a subset of 13 genotypes was grown in a low- and high-P field until maturity at ICRISAT-Samanko in Mali, WA. Root samples were taken at 38 days from the pot trial plants and at flowering time in the field trials. Shoot biomass was analyzed for P concentration and dry matter yield. GWAS was conducted for shoot-P-content and AMF-RC.

Results Significant genotypic variation was observed for AMF-RC, but the repeatability estimates were only low ($w^2=0.15$ at 38 days) to moderate ($w^2=0.54$–0.56 at flowering time). AMF-RC was significantly higher in low-P versus high-P field conditions. Large residual variation was observed for AMF-RC in both pot and field trials. None of the genotypic groups, contrasting for selection history, race and grain yield performance across multiple field trials, differed significantly for AMF-RC. AMF-RC showed no or negative relationships to shoot-P-content and grain yield, irrespective of soil-P level or plant developmental stage. AMF-RC at 38 days was significantly correlated ($r=67^{**}$) to AMF-RC at flowering. However, GWAS did not detect significant genomic regions for AMF-RC but did for shoot-P content.

Conclusion Although genetic differences for AMF-RC were detected, the trait appears to be highly polygenic. Genotypic selection for higher AMF-RC in WA sorghums is not promising due to the low heritability and the lack of positive relationships with P acquisition.

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Introduction

Sorghum (*Sorghum bicolor L.* Moench), a staple crop of Western Africa (WA), is mostly cultivated under low-input cropping conditions in WA. The major limiting factor for sorghum growth is soil phosphorus (P) availability in WA (Buerkert et al. 2001; Leiser et al. 2012). WA sorghum is generally well adapted to low P soil conditions and shows a large genetic diversity for agronomic P-efficiency displayed as grain yield productivity under low P soil conditions (Leiser et al. 2012). Furthermore, substantial genetic variation for P-uptake and internal P-use efficiency was observed in WA sorghum grown under P-limited conditions (Leiser et al. 2014b). Although both P-uptake and internal P-use efficiency contributed strongly to P-efficiency under low P conditions, a higher contribution to final grain yield production under P-limited conditions could be attributed to P-uptake traits (Leiser et al. 2015). For many crops it is known that several root mechanisms are directly or indirectly involved in P acquisition from the soil (Lynch 2011). Symbiosis with arbuscular mycorrhizal fungi (AMF) is one major plant adaptation to acquire P from soils (Smith et al. 1992). This symbiosis is thought to be particularly important in low-input cropping, such as sorghum-based cropping systems in WA (Bagayoko et al. 2001). The high diversity of AMF species in these low-input systems of WA (Friberg 2001) points to a potentially important role of mycorrhizal symbiosis for sorghum productivity.

Farmers in the Sudano-Sahelian region of WA currently cultivate traditional landrace varieties as well as newly bred varieties, developed by introgressing exotic germplasm into local landrace materials. Although both germplasm groups possess a large genotypic variation for P-uptake traits, the photoperiod sensitive landrace genotypes show on average a higher P-uptake capacity under P-limited conditions (Leiser et al. 2014b). As reported for wheat (Hetrick et al. 1992), a higher AMF root colonization (AMF-RC) of these landrace varieties might be the driving force for the observed higher P-uptake under P-limited conditions. To date there is no knowledge on the differences among germplasm groups in sorghum for their AMF-RC and how AMF-RC is related to P-uptake under P-limited and P-fertilized field conditions.

The large genotypic variation of AM fungal symbiosis observed within several crop species (Krishna et al. 1985; Baon et al. 1993; Kaeppler et al. 2000; Galvan et al. 2007; Smith et al. 2009; An et al. 2010; Hildermann et al. 2010; Singh et al. 2012) bears also great potential to identify WA sorghum genotypes which show higher AMF-RC under P-limited soil conditions. Despite the extensive cultivation of sorghum in WA under P-limited conditions, so far no study has examined the genotypic variation of AMF-RC of sorghum under P-fertilized and unfertilized conditions and the potential implications for sorghum selection for grain yield production under P-limited conditions.

Although genotypic variation for AMF-RC has been shown to increase in some cases P uptake (Smith et al. 2009), crop breeders are very hesitant to assess AMF-RC intensity due to the great amount of effort required to evaluate this root trait. Molecular markers associated to higher AMF-RC could be of great use to enable AMF-RC evaluation to be applied at large scale in a breeding program. Kaeppler et al. (2000) found one quantitative trait locus (QTL) associated to mycorrhizal responsiveness of maize plants grown under P-limited conditions. For sorghum there is currently no knowledge on the genetics underlying AMF-RC.

The objective of this study was to determine whether sorghum breeding programs should target higher colonization by AMF in order to enhance sorghum growth under P-limited conditions. Our specific objectives were: (i) characterize the genotypic variation for AMF-RC at two growth stages in a diverse panel of WA sorghums, (ii) determine the relationship of AMF-RC to plant performance under P-fertilized and unfertilized conditions at two growth stages and (iii) assess the genetics underlying AMF-RC using genome-wide association mapping in a panel of WA sorghums.

Materials and methods

Genotypes

A total of 187 sorghum genotypes from six West and Central African countries, consisting of researcher-bred and landrace varieties representing eight racial groups (Guinea, Durra, Caudatum and five intermediate groups; Suppl. Table 1) and differing degrees of photoperiod sensitivity and stem internode lengths were used in this study. Sixty-six out of the 187 genotypes could be

assigned to be of landrace or researcher bred origin based on their pedigree and selection history (Leiser et al. 2014b). Further details on the genotypes and population genetic parameters of the entire set are described in Leiser et al. (2014a).

Pot experiment (PEx)

The entire set of 187 genotypes was grown for 38 days in a pot experiment at the Samanko (12° 31′ N, 8° 4′ W) station of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in 2011. The experiment was laid out as α-design with four complete replications and incomplete blocks consisting of eight pots. The 10 l plastic pots were filled with homogenized top soil collected from an acidic P-deficient field (fine loamy Ferralsol with C:Si:S=25:50:25, pH_{H2O} 5.2, 5.2 mg Bray-P kg^{-1}soil, 12 % exchangeable acidity of CEC_{eff}) used for sorghum cultivation during the previous 3 years. Neither NPK fertilizer nor artificial mycorrhiza inoculate were applied. The soil was assessed for its natural mycorrhiza diversity and abundance microscopically based on morphological criteria (Oehl et al. 2003). Two untreated seeds were sown on 14 October 2011 in each of five central holes at 1.5 cm depth and covered with sand. The pots were watered each alternate day to field capacity. Seedlings were thinned to two plants per pot 14 days after sowing. Germination rate was very low in some pots with only one seedling surviving. Also some genotypes tillered profusely due to the colder nights (average min Temp. 17.5 °C) and the shorter photoperiod (~1 h shorter day length in Oct vs June/July) compared to the normal growing conditions of sorghum in a June/July planting at the beginning of the rainy season. Hence the number of plants and tillers were considered in the analysis of all biomass related traits. All plants were treated 12 and 18 days after sowing with lamda-cyhalothrine and acetamiprid to prevent damage by shoot fly (*Atherigona soccata*). Fresh shoot biomass was harvested and tiller number recorded. The shoot biomass samples were dried at 40–50 °C for several days until no more weight changes were observed. The dry biomass samples were ground and analyzed for their P concentration using an inductive coupled plasma optical emission spectrometer (ICP-OES) as described in VDLUFA (2011). Plants were uprooted and the soil washed from the roots with water immediately after shoot biomass harvest. Fine roots were stripped off the crown roots and samples were

stored in a mixture of 70 % ethanol and 80 % acidic acid (6:1). AMF-RC was assessed on these root samples by staining, based on Phillips and Hayman (1970) and Koske and Gemma (1989), and counting mycorrhizal roots using the modified gridline intersection method (Giovannetti and Mosse 1980).

Field experiment (FEx)

Thirteen genotypes for field experimentation were selected to represent the range of racial class, zone of adaptation and AMF-RC in PEx present in the total set of 187 genotypes. The field trials were sown on 16 June 2012 at Samanko, Mali, WA, i.e. at the same ICRISAT station as used for the PEx. Total amount of rain between date of sowing and root sampling was 893 mm and the temperature ranged from min 20 °C at night to max 42 °C during the day. Two separate trials were planted in adjacent fields, one with P fertilization (denoted "+P") and one without (denoted "–P"). The +P field was fertilized with 200 kg ha^{-1} diammonium phosphate (18-46-0) as basal fertilizer and urea (100 kg ha^{-1}) (46-0-0) as top dressing. The –P field received only topdressing with urea at rates that gave equivalent units of nitrogen as received by the +P field. The –P field was cultivated with sorghum for 5 years with zero P fertilization, was fallowed for more than 3 years preceding 2005 and had the following soil properties: fine loamy Ferralsol with C:Si:S=25:50:25, 4.6 mg Bray-P kg^{-1}soil, 124 mg P kg^{-1}soil, 0.17 % C_{org} and pH_{H2O} 6.0. The +P field was cultivated with sorghum for 4 years and had the following soil properties: fine loamy Ferralsol with C:Si:S=25:50:25, 28.5 mg Bray-P kg^{-1}soil, 268 mg P kg^{-1}soil, 0.36 % C_{org} and pH_{H2O} 5.1. Each trial consisted of 13 genotypes sown in a randomized complete block design with four complete replicates. Plots consisted of two three-meter rows with 75 cm distance between rows and 30 cm between hills within rows. Hills were thinned to two plants, resulting in a total of ~9.8 plants m^{-2}. A single border row planted with a medium tall, medium late maturing genotype separated each test plot to minimize neighbor effects. Shortly after flowering stage, four root samples from each plot were taken with an auger (diameter 3 cm) at a depth of 0–20 cm to assess AMF-RC. Each sample comprised two plants standing in one hill; hence eight plants per plot were sampled. The auger was inserted into the soil underneath each hill with an angle of 45° to sample a wide variety of root maturity stages. Soil was

washed off the samples and the remaining fine roots were stored in a mixture of 70 % ethanol and 80 % acidic acid (6:1). AMF-RC assessment was conducted with the same procedure as described above for PEx. Each plot was harvested at physiological maturity, with panicles and stover samples dried for several weeks in the sun until no weight changes were observed. Grain samples as well as ground stover samples were analysed for their P concentration using an inductive coupled plasma optical emission spectrometer (ICP-OES) as described in VDLUFA (2011).

Phenotypic data analysis

Each trait in PEx was separately analyzed using a REML-mixed model considering genotypes and replications as fixed effects and incomplete blocks nested within replications as random effects. The analysis was conducted in Genstat 17 (www.vsni.de). Genotypic variance components and repeatabilities (w^2, broad sense heritability) were estimated using the same model but taking genotypes as random effects. Repeatability was calculated with an adjusted formula for unbalanced experimental designs (Piepho and Möhring 2007, Eq: 19). The genetic coefficient of variation (GCV) was calculated by dividing the square root of the genetic variance component by the grand mean.

Each trait in FEx was first separately analyzed for each P-fertility treatment level using a REML-mixed model considering genotypes and replicates as fixed. Repeatability and GCV were calculated following the same procedure as for PEx. Furthermore each trait in FEx was also subjected to an analysis of variance (ANOVA) across both treatments modeling both main effects (genotype and phosphorus) and their interaction (genotype-by-phosphorus) and the replications nested within each treatment level. In order to calculate the broad sense heritabilities (h^2) for each trait across both treatments, a linear mixed model was modeled with genotypes as random and P-level and replication as fixed effects. The estimated variance components for genotype, genotype-by-P-level and for the residual were used to calculate h^2 with the following formula:

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{GP}^2}{2} + \frac{\sigma_{error}^2}{8}},$$

where σ_G^2 is the genetic variance component, σ_{GP}^2 is the genotype-by-P-level interaction variance component and σ_{error}^2 is the variance component of the residual.

The adjusted means of AMF-RC from PEx were regressed on the adjusted means from FEx using a linear regression analysis to determine the potential of AMF-RC at an early stage to predict AMF-RC at a later maturity stage. Due to the rather low $w2$ estimates, especially in PEx, we used the raw pot/plot values of AMF-RC and evaluated their linear predictive ability for P content in total above ground biomass (PSTBM, PBMY) in the respective pot/plot by running a linear regression analysis.

Genetic data analysis

Total genomic DNA was extracted from a single 20 day old plant of each line by using DNeasy Plant Mini Kit (QIAGEN). Genotyping by sequencing (GBS) was conducted following Elshire et al. (2011), using the enzyme ApeKI. The sequenced tags were aligned to the sorghum reference genome BTx623 v1.0 (Paterson et al. 2009) and only tags with at least 10× coverage were retained. Ambiguous or heterozygous sites were set as missing SNPs and finally imputed with all other missing SNPs using NPUTE (Roberts et al. 2007) for each chromosome separately. Imputation accuracy was on average above 96 %. In total 308 623 SNPs were retrieved. After filtering for 5 % minor allele frequency (MAF) using TASSEL, 220 934 SNPs were retained and used for further analysis.

The AMF-RC and PSTBM best linear unbiased estimates (BLUEs) estimated in PEx were used in a genome-wide association study (GWAS). GWAS was carried out in R using the package GenABEL (Aulchenko et al. 2007) running several models using the polygenic function correcting only for kinship or for kinship and population structure using either one, two, three, five or ten principal components (PCAs). The PCAs were calculated using all SNPs in SNPrelate (Zheng et al. 2012). Kinship among genotypes was calculated within GenABEL. The best GWAS model was chosen based on the lambda estimate of the quantile-quantile plots of the expected versus the observed p-values, thus having the lowest genome-wide inflation. For AMF-RC we observed an overcorrection (lambda <1), hence we used a naïve model, not correcting for any population structure. This model resulted in the best model fit. For PSTBM, a model using

K alone lead to the model fit and lowest lambda estimate. Multiple testing was corrected using a Bonferroni threshold of $p < 0.05$ (p-value/#SNPs). Possible causal changes of associated SNPs were tested by the Variant effect Predictor of the www.gramene.org website using the sorghum genome v.1.0. Genes in close vicinity to the detected SNPs and their possible functions were retrieved from the www.phytozome.com website using the sorghum genome v.1.4.

Results

AMF-diversity and abundance

A total of 33 AMF species (morphospecies) of 12 genera were detected by morphological analysis in two samples of soil from the field where FEx was conducted and from which soil was taken for PEx (Fig. 1). On average there were 13 spores per gram of soil. The predominant genera were Glomus (42 %), Acaulospora (17 %) and Claroideoglomus (17 %).

Genotypic variation of AMF-RC and agronomic traits

Significant genotypic variation was observed for AMF-RC at 38 days after sowing in the –P pot experiment (PEx) and at flowering under both –P and +P conditions in the field experiments (FEx, Table 1). Repeatabilities estimated for AMF-RC were low at the early stage of

plant development ($w^2 = 0.15$) and intermediate ($w^2 = 0.54$ to 0.56) at the later stage. A twofold genotypic range for AMF-RC with a grand mean of 55.4 % was detected in PEx. AMF-RC mean values in FEx were lower but the genotypic range was larger than in PEx. The AMF-RC mean observed in the –P field trial (27.6 %) was significantly higher than that observed in the +P trial (16.8 %) (Tables 1 and 2, Fig. 2). Single tiller biomass (STBM) and P content of single tiller biomass (PSTBM) in PEx showed large and highly significant genotypic variation with moderate repeatability estimates. Likewise, large and highly significant genotypic variations and high repeatabilities were observed in both P-treatments of FEx for grain yield (GY), stover yield (SY) and total biomass yield (BMY) as well as for total P content in BMY (PBMY). Total P-uptake in −P was only about half that of the +P trial, while –P BMY was only reduced by ~20 % relative to +P (Table 1).

Combined analysis of variance of AMF-RC over the –P and +P field trials revealed significant main effects for P-treatment and genotypes and weakly significant ($p < 0.1$) genotype-by-P interaction (Table 2). The high error coefficient of variation (48 %) and the low heritability estimate ($h^2 = 0.26$) reflect the moderate repeatability and GCV estimates of the single trial analyses. The very large variation of AMF-RC within each genotype and treatment can be also seen in the generally large ranges (between 1st and 3rd quartile) in the boxplots shown in Fig. 2. The two genotypes with the highest mean AMF-RC in –P conditions and the largest increase

Fig. 1 Soil mycorrhiza diversity, as % of spores of each of 12 genera in the –P soil used in the pot trial (PEx) taken from the –P field of the –P field trial (FEx)
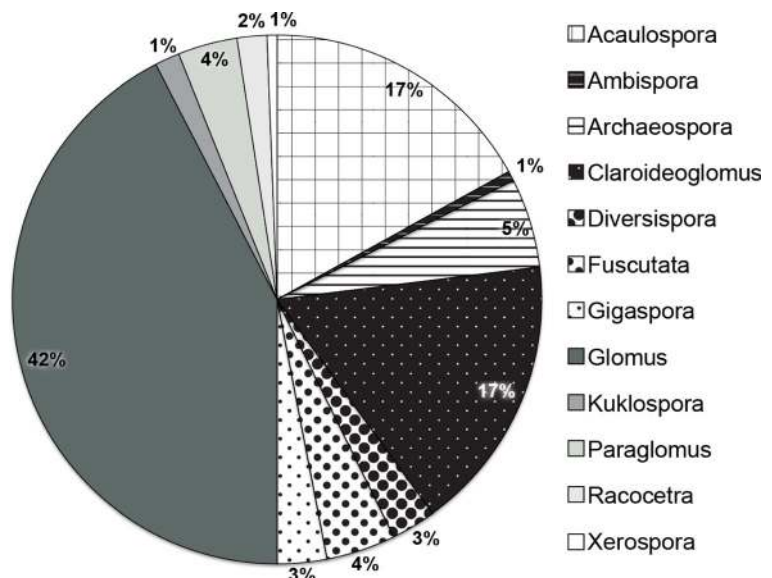
Table 1 Minimum, maximum and mean of adjusted means, significance of genetic variance, repeatability (w^2) and genetic coefficient of variation (GCV) for three traits in the –P pot trial (PEx) evaluating 187 genotypes and five traits in –P and +P field conditions (FEx) using 13 genotypes

Trial	P-level	Trait[a]	Unit	Min	Max	Mean	σ_G^2	w^2	GCV
PEx	–P	AMF-RC	%	38.54	72.86	55.44	*	0.15	4.54
PEx	–P	STBM	g S^{-1}	0.31	2.21	0.89	***	0.57	27.62
PEx	–P	PSTBM	mg S^{-1}	0.23	5.56	1.54	***	0.40	29.90
FEx	–P	AMF-RC	%	17.91	55.63	27.61	*	0.54	25.72
FEx	–P	GY	g m^{-2}	65.53	242.02	140.90	***	0.74	29.91
FEx	–P	SY	g m^{-2}	305.29	848.24	468.00	***	0.93	41.56
FEx	–P	BMY	g m^{-2}	423.43	1090.26	613.90	***	0.88	34.25
FEx	–P	PBMY	mg m^{-2}	254.92	787.60	461.50	***	0.82	29.64
FEx	+P	AMF-RC	%	9.79	31.53	16.78	*	0.56	25.48
FEx	+P	GY	g m^{-2}	80.74	433.29	196.10	***	0.89	42.72
FEx	+P	SY	g m^{-2}	285.64	994.15	535.60	***	0.98	45.13
FEx	+P	BMY	g m^{-2}	428.86	1427.44	736.50	***	0.97	39.80
FEx	+P	PBMY	mg m^{-2}	562.18	1615.07	869.90	***	0.87	30.51

*, *** Significant at the 0.05 and 0.001 probability levels, respectively

[a] Arbuscular mycorrhiza fungal root colonization (AMF-RC), single tiller biomass (STBM), total P content of single tiller biomass (PSTBM), grain yield (GY), stover yield (SY), total biomass yield (BMY) and total P content in biomass (PBMY)

in –P relative to +P conditions were Lata and Tiandougou (Fig. 2). Although most genotypes had higher AMF-RC values in –P compared to +P conditions, NafalenP6 and MDK had higher values in +P (Fig. 2).

Examination of possible relationships between AMF_RC levels and racial groups or breeding history of accessions in our sorghum panel was conducted by classifying the 187 genotypes using the racial classification system of Harlan and de Wet (1972) and a subset of the panel having Sudanian zone origin (66 genotypes) was also classified based on selection history (landrace vs breeding line). The adjusted

means of AMF-RC of these genotype groups were analyzed to detect presence of significant differences. Although the landrace variety group showed slightly higher AMF-RC values compared to the breeding lines, but the difference was only weakly significant ($p = 0.1$) (Fig. 3). Similarly, the eight racial groups did not differ significantly ($p = 0.2$), in part due to highly variable number of entries per racial group. The racial group with the numerically highest AMF-RC level was the Guinea-Durra interracial genotype group, consisting of only four entries.

Additionally, possible relationship between AMF-RC levels and grain yield performance in

Table 2 Mean squares, the significance level of each treatment, the error coefficient of variation (CV%) from ANOVA and the broad sense heritability (h^2) for arbuscular mycorrhiza fungal root colonization (AMF-RC), stover yield (SY), grain yield (GY), total biomass yield (BMY) and total P content in biomass (PBMY) of 13 sorghum genotypes evaluated in –P and +P field conditions

Trait	Units	Phosphorus	Genotype	GxP	CV%	h^2
AMF-RC	%	3054.2*	292.6**	214.8+	48.6	0.26
SY	g m^{-2}	113,676+	382,245***	20,537*	18.8	0.94
GY	g m^{-2}	79,233**	35,184***	5855*	32.3	0.83
BMY	g m^{-2}	374,383*	516,903***	39,427*	19.8	0.92
PBMY	mg m^{-2}	4,441,851***	365,940***	50662^{ns}	26.3	0.86

+, *, **, *** = F-statistics of respective mean squares significant at p-level<0.1, 0.05, 0.01, 0.001, respectively
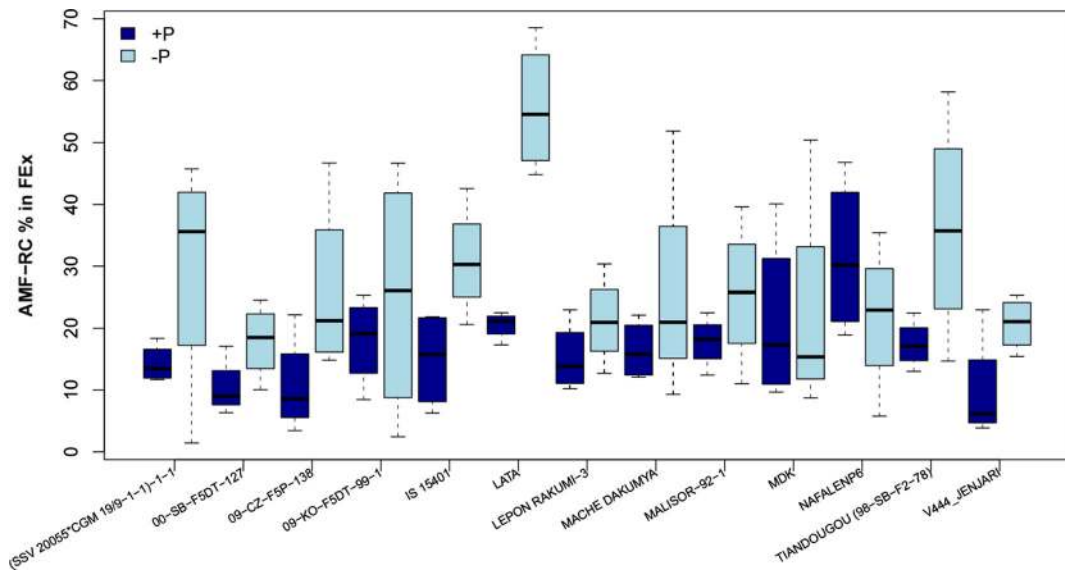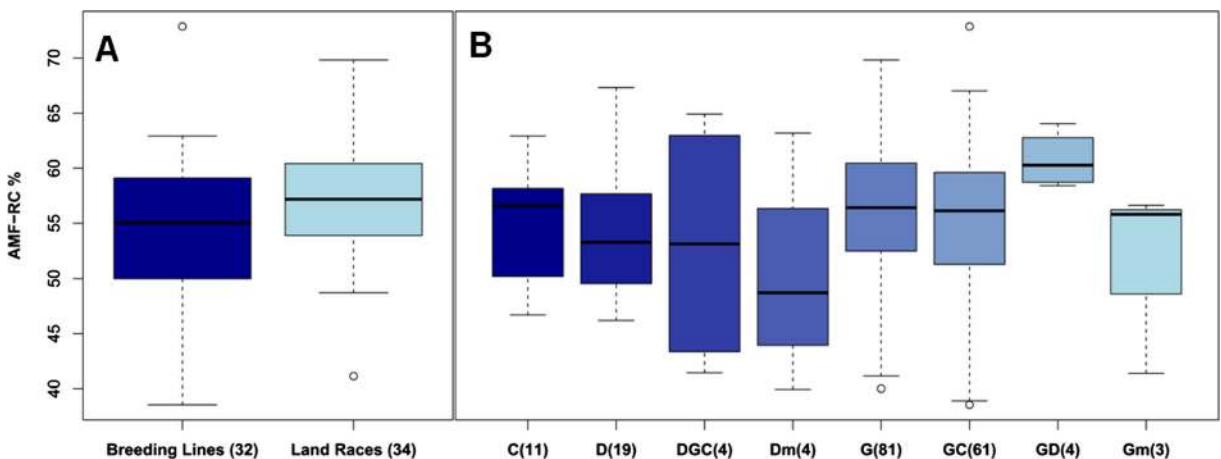
Fig. 2 *Boxplot* of arbuscular mycorrhiza fungal root colonization (AMF-RC %) of 13 sorghum genotypes at flowering time in +P (*dark*) and –P (*light*) field conditions

independent environments was examined by grouping genotypes based on their grain yield performance across 29 yield trials in WA (for details see Leiser et al. 2014a). Genotype groups corresponding to the four quartiles for grain yield performance a) across 15 –P environments, b) across 14 +P environments and c) for grain yield ratios (−P/+P) over 13 pairs of –P and +P environments were compared. No significant differences for

AMF-RC in PEx were observed among the four quartile groups for any of the grain yield performance criteria (Suppl. File 1).

Furthermore the genotypic AMF-RC adjusted means of PEx were also regressed against the grain yield data obtained from yield trials across 15 –P environments in WA. No significant relationship between AMF-RC and GY in –P conditions could be detected (Suppl. File 2).
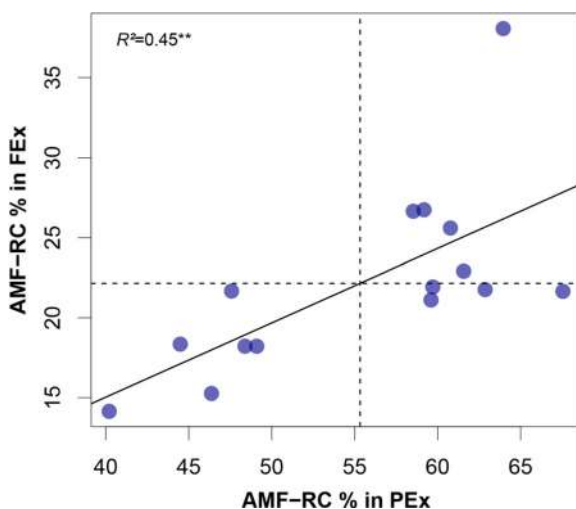

Fig. 3 Boxplot of adjusted means of arbuscular mycorrhiza fungal root colonization (AMF-RC %) of genotypes grouped based on their selection history (**a**) and based on their morphological racial classification (**b**) at 38 days after sowing in a low P soil pot trial. No significant grouping effect could be detected in a two-sided *t*-test (**a**) or in a one-way ANOVA (**b**). *Numbers in brackets* indicate the number of genotypes/values in each *group/box*. Genotype races in B are: Caudatum (C), Durra (D), Durra-Guinea-Caudatum (DGC), Durra membranaceum (Dm), Guinea (G), Guinea-Caudatum (GC), Guinea-Durra (GD) and Guinea margaretiferum (Gm). Genotypes in A are a subset with known selection history from all 187 genotypes

Correlations

AMF-RC adjusted means of the 13 genotypes that were common between PEx and FEx were subjected to a linear regression analysis in order to evaluate the relationship of AMF-RC at flowering stage in the field relative to AMF-RC at early plant developmental stage in pots. AMF-RC at an early stage had a moderate predictive ability of AMF-RC at flowering and could account for 45 % ($r=0.67$**) of the variation (Fig. 4). Mean AMF-RC of the young roots in PEx was more than twofold compared to AMF-RC of the old roots at flowering stage in FEx.

Correlations between AMF-RC and total P content in aboveground biomass were examined by regressing P content in the above ground biomass on AMF-RC values in both the PEx and FEx. A significant negative relationship ($p=-31$***, Fig. 5a) was detected between P content of single tiller biomass (PSTBM) and AMF-RC in PEx. Similarly, a negative, but weaker, correlation ($p=-0.22$*) was also seen between AMF-RC and P content of total biomass (PBMY) when the –P and the+P FEx trials were analyzed together (Fig. 5b). Analysis of the FEx trials separately by P levels (Fig. 5c, d) revealed no significant relationship between AMF-RC and PBMY in either –P or +P conditions.

GWAS for AMF-RC and P content of single tiller biomass (PSTBM)

A genome wide association study was conducted for the AMF-RC and PSTBM traits evaluated in PEx. No SNP marker significantly ($p<0.05$) associated with AMF-RC could be detected (Fig. 6a). However, three SNPs on chromosome 7, 8 and 10 showed $-\log_{10} p$-values of >4 which, although far below the Bonferroni corrected significance level ($-\log_{10} p=$ 6.64), could indicate possible genes involved in AMF-RC at low P conditions in sorghum, with each SNPs explaining around 8 % of the genotypic variation.

SNPs significantly associated with PSTBM were found on independent regions of chromosome 2, one on the short arm and another on the long arm, and a third region with highly significant association to PSTBM was detected on chromosome 3 (Fig. 6b). The two most significant SNPs on chromosome 2 explained 15-18 % of the genotypic variation, while the most significant SNP on chromosome 3 explained 14 %. Thus SNPs detected for PSTBM and those possibly detected for AMF-RC did not co-locate on common chromosomes, which corresponds to our observation of only a weak correlation between PSTBM and AMF-RC (Fig. 5a).
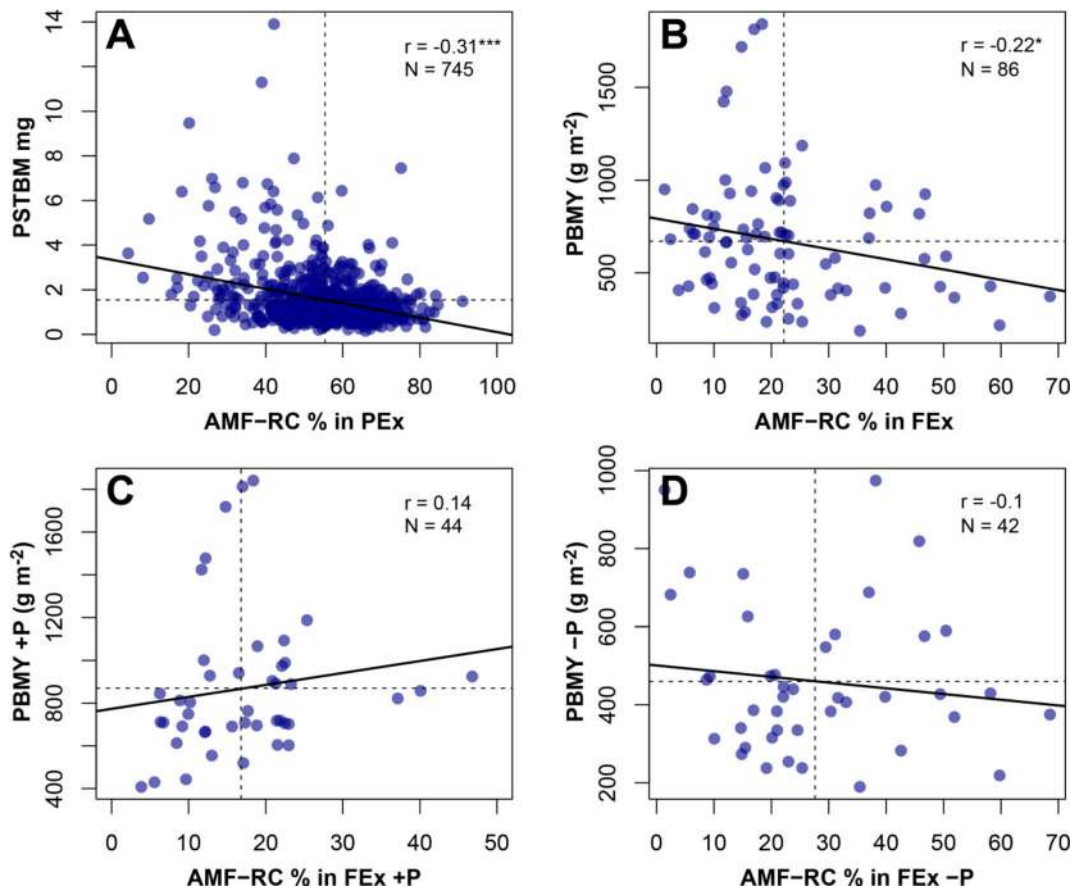
Discussion

High AMF diversity and abundance in low P soils

Malian soils, especially low-input agricultural soils, generally have high AMF diversity (Friberg 2001). Observations in Benin showed that AMF diversity and AMF spore abundance increases as the level of soil fertility declines (Tchabi et al. 2008). The high AMF diversity (33 species of 12 genera) and high AMF abundance (13 spores per gram) observed in our long-term low-P soil conforms to this pattern. The predominance of Glomus, followed by the Acaulospora genera, in our low-P soil from Mali was similar to the results from Benin reported by Tchabi et al. (2008) and for other agricultural soils outside of WA (Oehl et al. 2004; Wang et al. 2008).

The high AMF diversity and increased abundance of AMF with decreasing soil P levels in agricultural soils in WA suggests that AMF plays a role in the

Fig. 4 Scatter plot of adjusted means for AMF-RC % at early plant growth (38 days) in –P soil in pots (PEx) and at flowering time across –P and +P field conditions (FEx). *Dashed lines* indicate means and *solid line* indicates linear regression between the plotted traits

Fig. 5 Scatter plots of phenotypic data (using raw pot/plot values) of total P content in harvested above ground biomass and AMF-RC at an early growth stage (38 days) in –P soil conditions in pots (**a**), at flowering time across both +P and –P field conditions (**b**) and at flowering time in +P (**c**) and –P (**d**) field conditions. *Dashed lines* indicate means and *solid lines* indicate linear regression between the plotted traits. ***,* indicate significance level of correlation coefficient (r) at *p<0.001* and *p<0.05*, respectively. N shows number of values subjected to analysis

low-input agricultural systems of WA. The diversity and abundance of AMF in the low–P soil used in our study suggests that there was sufficient natural AMF inoculation to enable effective genotype screening with high levels of AMF-RC (Smith and Smith 1981; Krishna et al. 1985). We did not conduct separate evaluations in sterilized and artificially infested soils, as is done in many smaller AMF-RC studies, since we used both field based and pot based evaluations of an unprecedented large number of sorghum genotypes tested in larger quantities of soil. Additionally, our main aim was to assess the potential of using genotypic differences for natural AMF-RC as a selection criterion for breeding low-P adapted sorghum genotypes for WA and not the relative response of sorghum to AMF treatment versus non-treated sterilized soil conditions.

Genetic variation for AMF-RC in WA sorghums

Our observation of significant genotypic variation for AMF-RC in WA sorghum corresponds to results from onion, wheat, maize and pearl millet (Krishna et al. 1985; Galvan et al. 2007; An et al. 2010; Hildermann et al. 2010; Singh et al. 2012) and confirms the existence of genotypic differences for AMF-RC across species and environments. Nevertheless, these genotypic differences are small (low GCV), not highly repeatable (w^2= 0.15-0.56), show low heritability estimates over environments (h^2=0.13-0.26; see also Kaeppler et al. (2000)) and are therefore of little value as selection criteria for plant breeding programs (Galvan et al. 2007).

Hetrick et al. (1992) reported higher AMF-RC for old US wheat varieties compared to modern varieties, but these results could not be confirmed in two other wheat
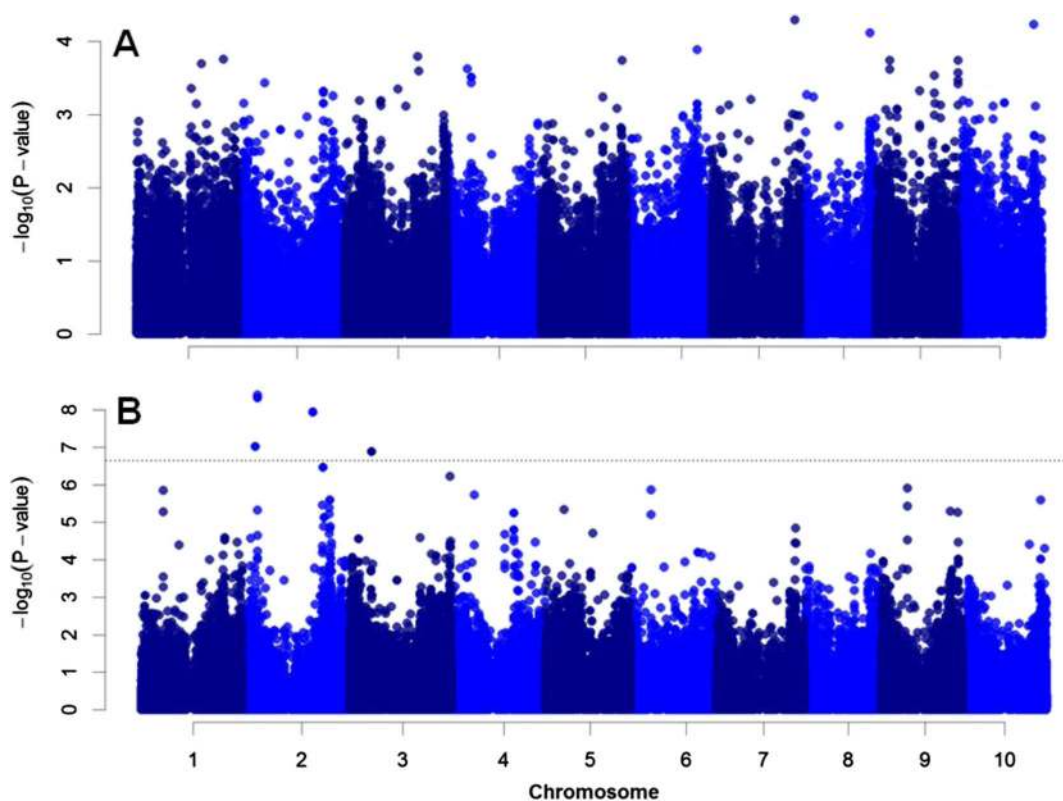
Fig. 6 Manhattan plot for arbuscular mycorrhiza fungal root colonization (AMF-RC %; A) and total P content of single tiller biomass (PSTBM; B) BLUEs evaluated in PEx of 187 genotypes genotyped with ~200 k SNPs with p-values shown on a $\log_{10}$ scale and Bonferroni threshold at $p<0.05$ indicated with *dashed line*

and maize studies (An et al. 2010; Hildermann et al. 2010). Our study's comparisons of AMF-RC levels for genotype groups based on selection history, race and grain yield performance across multiple field trials did not detect any significant differences. Our lack of differences between contrasting groups may be the consequence of limited plant-available soil-P being a common stress across WA sorghum cropping systems. The low heritability and lack of clear groups as superior sources for AMF-RC hinder exploiting AMF-RC in WA sorghum breeding.

Impact of AMF-RC on P-uptake and grain yield under P limitation

The impact of AMF-RC on plant growth and P uptake has been reported to range from positive to negative under P-limited conditions. However, studies commonly used sterilized and re-inoculated soils, with AMF as a treatment factor and often observed a positive relationship between AMF-RC and plant response to AMF

treatment in biomass production (Gange and Ayres 1999). In contrast, many studies relating AMF-RC and plant growth in the same treatment (e.g. +AMF-treatment) or as in our study without soil sterilization and artificial inoculation, most frequently observed no response or a negative impact of AMF-RC on plant growth and P-uptake (Krishna et al. 1985; Baon et al. 1993; Kaeppler et al. 2000; Bagayoko et al. 2001; Ryan et al. 2002; Ryan and Angus 2003; Kaeppler 2008; Hildermann et al. 2010; Smith and Smith 2012). Our negative relationship between PSTBM and AMF-RC at an early plant developmental stage and the non-significant correlation between AMF-RC and PBMS at flowering and grain yield across multiple environments therefore confirm these findings, and warn that selection for higher AMF-RC will not necessarily lead to enhanced performance in low-P soils of WA. Neutral or even negative effects of AMF-RC on plant growth have long been postulated to be caused by a somewhat parasitic carbon drain from the plant to AMF ineffective in supplying sufficient P to the host plant (Johnson et al.

1997) and is still debated (Smith and Smith 2012). Smith et al. (2009) showed that direct root-induced P uptake and the symbiotic (mycorrhizal) P-uptake system of plants are not additive traits. In the presence of AMF, primary root P-uptake was shown to be down-regulated by the plant and mycorrhizal P uptake can account for more than 80 % of the total P acquisition by the host plant (Smith et al. 2003, 2004; Smith and Smith 2012). Hence, a negative or neutral relationship of AMF-RC and P-uptake is possible in situations of AM colonization due to a reduced direct root P uptake that is not sufficiently compensated by delivery of P via the AM pathway in the less colonized roots (Smith et al. 2009; Smith and Smith 2012). The same holds true on highly P-fixing soils (e.g. ferralsols) with insufficiently plant-available soluble-P levels, representing the major source for P acquisition of AM-associations.

Our study evaluated a genetically diverse sorghum collection from WA. Different adaptation strategies appeared to be used by these genotypes, some with high P uptake, others with high P utilization efficiency, but both capable of producing high grain yields under P-limited conditions (Leiser et al. 2014b). Therefore it is not surprising that no significant relationship between AMF-RC and grain yield performance was found among these genotypes. Furthermore, we conducted our genotype screening in a naturally infected low P soil, which showed a wide variety of AMF species. The different AMF species might interplay and have an effect which is still not clear (Smith and Smith 2012), hence leading to AMF species specific genotype responses in our diverse set of material as shown in wheat (Mao et al. 2013).

Controlled screening in artificially inoculated soils with a limited number of AMF genera has been argued to be the only option for selecting genotypes with positive response specifically to AMF. Kaeppler (2008) and Singh et al. (2012) concluded that such a selection system could not obtain genotypes with stable responsiveness over diverse environments. Tillage and environmental differences in AMF abundance and diversity (Gange and Ayres 1999; Simpson et al. 2011; Ryan and Kirkegaard 2012) are the two major factors, with tillage disrupting the hyphae system and the environmental variability of soil AMF populations preventing consistent specific AMF responses. Another, although similar idea was proposed by Sawers et al. (2010) and Fester and Sawers (2011), which suggests that breeders should select genotypes which are not highly dependent on AMF (e.g. well performing without AMF in low P soils) but still have a high responsiveness to AMF inoculation. Although this strategy would be advisable for enhancing AMF-responsiveness, it still does not overcome the two major hurdles of genotype selection for AM fungal symbiosis, limited and species specific genetic variation and low heritability. Furthermore, evaluating genotypes in two conditions (−AMF, +AMF) would double the necessary resources or limit the number of genotypes tested, both leading to a lower response to selection.

Genomic regions for AMF-RC

Although we observed significant genotypic differences for AMF-RC among the 187 diverse sorghum genotypes from WA at 38 days after sowing, no genomic region could be detected which showed a highly significant association to AMF-RC. The power of detecting a significant quantitative trait locus (QTL) generally decreases with decreasing heritability and lower number of tested genotypes. The very low heritability estimate of 0.15 and the limited number of 187 genotypes highly limits the potential of detecting significant QTL in this study. Furthermore, it shows that AMF-RC is controlled by many different genomic regions and is mostly influenced by environmental factors. Although Kaeppler et al. (2000) found one significant QTL for AMF-RC in a bi-parental maize population, this QTL explained only 6.5 % of the phenotypic variation, pointing to a similar conclusion as in our study, that AMF-RC is a highly polygenic trait, which is mostly influenced by environmental factors and does not serve as a trait for direct crop improvement.

Even though we did not find any significant SNPs (above Bonferroni correction threshold) for AMF-RC, annotation of the top three SNPs on chromosome 7, 8 and 10, showed patterns of stress response of the sorghum plants to AMF. SNP S10_54993244 (p-value= 9.58^{-06}) was located in gene Sb10g025620, a transcription initiation factor (TFIID, subunit TAF5 (also component of histone acetyltransferase SAGA)). TAF5 (TATA binding protein-associated factor 5) is a subunit of TFIID and SAGA (Spt–Ada–Gcn5), which is involved in the transcription of mostly stress induced gene complexes (Timmers and Tora 2005). The identification of this SNP might indicate activation of stress induced genes upon mycorrhization. Furthermore, two putative Leucine Rich Repeat (LRR) genes (Sb08g019980 and

Sb08g019990) were found in proximity to S8_51025447 (p-value 5.14^{-05}, 10 kb downstream) and one putative LRR gene (Sb07g025500) was found in proximity to S7_60597044 (p-value 3.62^{-05}, 10 kb upstream). LRR domains are known to be associated with pathogen response/recognition by plant pathogen associated molecular patterns. Schnabel et al. (2005) and Amiour et al. (2006) identified a putative LRR-receptor-like kinase gene (SUNN/MtSYM12) involved in the autoregulation of mycorrhization and nodulation in *M. truncatula*. These patterns of stress due to AMF, might give some additional explanation for our negative correlation between AMF-RC and plant growth under P-limited conditions.

Genomic regions for shoot P content

Three highly significant genomic regions on chromosome 2 and one region on chromosome 3 were identified for PSTBM at 38 days after sowing in low P soils. In close vicinity (≥30 kb up/downstream) of the strongest associated SNP S2_10933736 (p-value=4.09^{-09}) we detected only genes with unknown annotated functions. Similar for SNP S2_51186774 (p-value=1.14^{-08}), at a distance of >10 kb downstream, we detected the gene Sb02g020830, predicted as TPX2 (targeting protein for XKLP2), which is known to be involved in mitosis and spindle formation (Evrard et al. 2009), but has no clear function or any indication of up-regulation during P stress. Both SNPs S2_9122222 (p-value=9.58^{-08}) and S3_16031350 (p-value=1.32^{-07}) were located within a gene. While SNP S3_16031350 caused a missense variation (amino acid change from valine to leucine) in gene Sb03g013160, which carries a CRS1 domain, with no clear relationship to P stress, SNP S2_9122222 was located in a none-coding region of gene Sb02g007140, which is annotated as UDP-glucosyltransferase. UDPs have been shown to be up-regulated during P stress in maize seedling roots (Lin et al. 2013). Two further SNPs, which were just below the Bonferroni correction threshold, showed gene annotations, which might confirm recent findings in rice and sorghum under P stress conditions. SNP S2_58597154 (p-value=3.49^{-07}) was in close vicinity (upstream <2 kb) to gene Sb02g024310. Sb02g024310 possesses two annotated domains, a U-box and a protein-kinase (Serine/threonine) domain. U-box domains are known from rice to be up-regulated during P-stress (Hur et al. 2012) and kinases have been associated with increased

root growth and biomass production in rice (Gamuyao et al. 2012) and sorghum (Hufnagel et al. 2014) under P limited conditions. SNP S3_73278352 (p-value= 5.89^{-07}) on chromosome 3, was in close vicinity to an already identified QTL for root surface area and fine root volume production of sorghum under P limited conditions (Hufnagel et al. 2014). These findings give further evidence that root growth in low P soils is of major importance for P-uptake under these harsh growing conditions.

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