

RESEARCH

Genetic Architecture of Phosphorus Use Efficiency in Tropical Maize Cultivated in a Low-P Soil

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ABSTRACT

Phosphorus (P) deficiency is a major limiting factor for crop production in several countries. A better understanding of the genetic components of P use efficiency (PUE) is required to improve crop performance in low-P soils. To date, no QTLs (quantitative trait loci) were mapped for PUE using grain yield and other late phenotypic data in tropical conditions. Thus, we evaluated the genetic architecture of PUE in tropical maize (*Zea mays* L.) using multiple interval mapping for design III in a population of 140 RILs (recombinant inbred lines) backcrossed with both parental lines. The parental lines contrasted for yield and for PUE, a phenotypic index that was further decomposed into P acquisition efficiency (PAE) and P utilization efficiency. Our results showed that dominance effects were more important than additive effects for explaining the variations in PUE and its components. Approximately 80% of the QTLs mapped for PAE co-localized with those for PUE, indicating that the efficiency in acquiring P is the main determinant of PUE in tropical maize. Also, QTLs for PUE and PAE were located near to candidate genes previously associated with root development. Thus, we present important information to guide breeding strategies for the development of maize cultivars more adapted to P deficiency.

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Abbreviations: GY, grain yield; LOD, logarithm of odds; MIM, multiple interval mapping; PAE, phosphorus acquisition efficiency; Psoil, phosphorus soil content; PUE, phosphorus use efficiency; PUTIL, phosphorus internal utilization efficiency; QTL, quantitative trait loci; RIL, recombinant inbred line; SDW, shoot dry weight.

MAIZE (*Zea mays* L.) is the most cultivated cereal in the world. In the last decade, corn production increased by about 40%, reaching a total of 840 million tons in 2011 (FAOSTAT, 2013). The growing demand for food and energy requires the incorporation of new areas into the agricultural sector as well as increases in crop yield (FAO, 2010).

Phosphorus (P) is one of the most consumed nutrients for crop production worldwide (Cordell et al., 2009) and the main mineral required for maize development in the Brazilian acid savannas (Fageria, 1998). Approximately half the world's agricultural lands are low in P (Lynch, 2011). The soils in those areas show high P fixation capacity, which elicits P deficiency by restricted P availability (Collins et al., 2008). Additionally, P fertilizers are costly and derive from nonrenewable sources (Vance et al., 2003; Cordell et al., 2009). Thus, there is an urgent need for research efforts aimed at the development of plants that are more efficient in P uptake as well as in the mechanisms of its utilization within the plant.

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Different concepts have been used to define nutrient use efficiency in plants (Parentoni et al., 2012). Moll et al. (1981) defined P use efficiency (PUE) as the ratio between grain yield and the amount of P supplied to the crop. This index was decomposed into components related to the plant's ability to acquire the nutrient from the soil (P acquisition efficiency, PAE) and the internal metabolic processes of this nutrient within the plant (P internal utilization efficiency, PUTIL). On the basis of these indexes, Parentoni and Souza Júnior (2008) showed that variations in PAE were more important than those in PUTIL for explaining the variability in PUE in tropical maize germplasm cultivated in low-P soils.

As P has low mobility in the soil, improving root development is expected to increase PAE, resulting in better PUE. In maize, genes controlling root development and root morphology have been cloned (Lim et al., 2005; Wen et al., 2005; Brady et al., 2006; Taramino et al., 2007; Hochholdinger et al., 2008), and some of these genes were differentially expressed in maize lines contrasting for PAE (de Sousa et al., 2012). Gamuyao et al. (2012) have shown that *Pstol1* (phosphorous-starvation tolerance 1), a gene encoding a protein kinase, enhances early root development in rice (*Oryza sativa* L.), improving P uptake and, ultimately, increasing grain yield under P deficiency. Thus, these results reinforce the hypothesis that molecular mechanisms affecting root development may also modulate PAE in maize.

Genetic analyses based on generation means in maize revealed that PUE is a complex trait with a prevalence of dominance over additive effects (Parentoni and Souza Júnior, 2008; Parentoni et al., 2010). Design III, proposed by Comstock and Robinson (1952), is an appropriated strategy to study the genetic architecture of PUE because of its ability to estimate the average degree of dominance of quantitative trait loci (QTLs). However, estimates of variance components may be biased in the presence of genetic linkage (Comstock and Robinson, 1952) and epistasis (Melchinger et al., 2007) among QTLs. The former is expected to reduce on several generations of selfing, such as in recombinant inbred lines (RILs). Garcia et al. (2008) developed a multiple interval mapping (MIM) model for design III that provides more reliable estimates of augmented additive and dominance effects and also accommodates epistatic interactions among QTLs. By analyzing both backcross progeny simultaneously, this model can be applied for segregating populations with any level of selfing (Garcia et al., 2008).

A few QTL studies have been performed for PUE in maize under field conditions (Chen et al., 2008, 2009, 2011). However, in these studies, the P efficiency indexes were calculated on the basis of the total weight of plants harvested on the 21st day after seedling emergence. Phosphorus efficiency indexes estimated on the basis of early traits may have limited application as indirect selection criteria in breeding programs, which will be highly dependent

on the correlation of such traits with grain yield. Quantitative trait loci for grain yield and its components evaluated in high- and low-P soils were mapped only in temperate conditions (Li et al., 2010), which may not be coincident with conditions in the tropics. Thus, we applied a very robust strategy to map QTLs using MIM for design III to dissect the genetic architecture of PUE and its components on the basis of grain yield in tropical maize cultivated under low-P soil. Our results bring new insights for breeding programs aiming to improve P use efficiency in maize.

MATERIAL AND METHODS

Genetic Material

We used a population composed of 140 RILs, which were backcrossed with both parental lines L3 and L22, following a modification of design III (Comstock and Robinson, 1952; Garcia et al., 2008). Parents and RILs were developed by Embrapa Maize and Sorghum, Sete Lagoas, Minas Gerais, Brazil. L3 and L22 were previously classified as P-efficient and P-inefficient lines, respectively (Parentoni et al., 2010).

Genetic Map

DNA was isolated from young leaves using the cetyltrimethylammonium bromide (CTAB) method (Saghai-Marouf et al., 1984). Initially, 60 polymorphic simple sequence repeat (SSR) markers were genotyped in the RIL population according to Ninamango-Cárdenas et al. (2003). Additionally, 332 single nucleotide polymorphisms were mapped in the population using Kompetitive Allele-Specific polymerase chain reaction, called KASP assay, from LGC Genomics (www.lgcgenomics.com, accessed 18 Apr. 2014). Sequence information and physical position of SNPs are available from Panzea (www.panzea.org/db/searches/webform/marker_search, accessed 18 Apr. 2014), whereas genetic information for SSRs is found at Maize-GDB (www.maizegdb.org/locus.php, accessed 28 Apr. 2014).

Sequence-tagged site markers to map genes associated with root morphology were developed on the basis of polymorphisms detected in the parents. For the *roothairless* gene (*rth1*) (Wen et al., 2005), a 22-bp indel was amplified using the primers 5'-TTGCCACGGCTGGCAAGAG-3' and 5'-GGCTCTGTAGCACGCCCTC-3' and resolved on silver-stained polyacrylamide gel according to Creste et al. (2001). The same strategy was used for the *brittle stalk-2-like protein 3* gene (*Bk2L3*) (Brady et al., 2006), which was revealed by the amplification of a 15-bp indel using the primer pair: 5'-GCTGGTTAGATCCCCCGCCCA-3' and 5'-GCACTG-GAGCCACCGACACTG-3'. The *rootless concerning crown and seminal roots* gene (*rtc3*) (Taramino et al., 2007) was genotyped as a cleaved amplified polymorphic sequence marker obtained after digestion with the restriction enzyme *AciI* of the amplified product of genomic DNA with the primers 5'-CGCGCCATAGCCCG-CAGTAA-3' and 5'-GATTGGCACGGGCGGTCAG-3' and visualized on silver-stained polyacrylamide gels.

Markers were tested for an expected segregation ratio of 1:1 using chi-square statistics ($p < 0.05$), corrected for multiple tests on the basis of Bonferroni's method. The genetic map was constructed using the MAPMAKER/EXP 3.0b software (Lincoln

et al., 1992), with a minimum logarithm of odds (LOD) of 3.0 and a maximum distance between adjacent markers of 40 cM. The Kosambi mapping function (Kosambi, 1944) was used to convert the recombination fractions into map distance.

Phenotypic Traits

Progenies backcrossed to parental lines were grown on five rectangular lattice trials with three replicates, in soil with low P availability. Each trial was composed of 28 RILs backcrossed to both parental lines, for a total of 56 progeny per lattice. Each plot consisted of a 4-m row, with 0.8 m between rows, and 20 plants per meter. Trials were conducted at Embrapa Maize and Sorghum, Brazil (latitude 19°27' S and 716 m above sea level) during the summer season of 2010–2011, in a clay, dark red Ferralsols under Cerrado vegetation. At sowing, 30 kg ha⁻¹ of N and 60 kg ha⁻¹ of K₂O were applied to the soil, and 30 d after planting, the soil was further supplemented with 90 kg ha⁻¹ of N, supplied as urea. The total P available in the soil between depths of 0 and 60 cm (Psoil) was 10.14 kg.ha⁻¹, as determined by chemical analysis.

Grain yield (GY) was measured by weighing the grains in each plot, adjusting the weight to the standard 130 g ka⁻¹ grain moisture and converting to kg ha⁻¹. Each shoot sample consisted of leaves, stalk, and ear husks of five representative plants from each plot. Grain and shoot samples were weighed before drying, and their dry matter was determined by drying until constant weight in a forced-air oven at 65°C. Subsequently, all samples were ground, homogenized, and subjected to a nitric perchloric acid digestion following P quantification with an inductively coupled argon plasma (ICP) emission spectrometry. The P content in the grain (Pg) or in the shoot (Ps) was calculated by multiplying grain or shoot dry weight, respectively, by the P concentration in the corresponding plant component. The total P content in the plant (Pt) was calculated as Pt = Pg + Ps. The PUE index for each plot was calculated according to Moll et al. (1981), using the formula PUE = PAE · PUTIL, where PUTIL = GY/Pt and PAE = Pt/Psoil.

Statistical Analysis

Analyses of variance were performed for all traits according to Comstock and Robinson (1952) using the statistical model:

$$y_{ijklw} = m + b_{k(lw)} + r_{l(w)} + s_w + g_{i(w)} + p_{j(w)} + gp_{ij(w)} + \bar{e}_{ijklw},$$

where y_{ijklw} is the observation in the progeny i backcrossed with the parental j in block k in the replication l and trial w ; m is general mean; $b_{k(lw)}$ is the block effect in replication l and trial w ($k = 1, \dots, 8$); $r_{l(w)}$ is the replication effect in trial w ($l = 1, \dots, 3$); s_w is the trial effect ($w = 1, \dots, 5$); $g_{i(w)}$ is the progeny effect ($i = 1, \dots, 140$); $p_{j(w)}$ is the inbred parents effect ($j = 1, 2$); $gp_{ij(w)}$ is the parent and progeny interaction effect; and \bar{e}_{ijklw} is the experimental error. All analyses were performed in the PROC GLM procedure of SAS (SAS Institute, 1999). The broad-sense heritability (\hat{h}^2) for each trait was estimated on a mean basis by $\hat{h}^2 = \hat{\sigma}_g^2 / (\hat{\sigma}_g^2 + \hat{\sigma}_e^2 / r)$ where $\hat{\sigma}_g^2$ is the genotypic variance among progeny, $\hat{\sigma}_e^2$ is the error variance, and r is the numbers of replicates (Hallauer et al., 2010). The phenotypic (\hat{r}_p) and genotypic correlation (\hat{r}_g) between traits were estimated using the GENES software (Cruz, 2013).

QTL Mapping

For QTL mapping, we used the adjusted means for experimental sources (blocks, replicates, and trials) of PUE indexes for 280 backcross progeny, using multiple interval mapping for design III (Garcia et al., 2008), following the statistical model:

$$Y_{ij} = \mu_j + \sum_{r=1}^m \alpha_r x_{ijr}^* + \sum_{r=1}^m \beta_r z_{ijr}^* + \sum_{r<s}^{t_1} \gamma_{rs} \omega_{ijrs}^* + \sum_{r<s}^{t_2} \delta_{rs} o_{ijrs}^* + e_{ij},$$

where Y_{ij} is the phenotypic mean of the progeny i ($i = 1, 2, \dots, 140$) backcrossed with the inbred parental j ($j = 1, 2$); μ_j is the general mean of all progeny backcrosses with parent j ; α_r is the regression coefficient for augmented additive effect (a^*) of QTL r ; β_r is the regression coefficient for augmented dominance effect (d^*) of QTL r ; γ_{rs} and δ_{rs} are the regression coefficients for interactions $aa + dd$ and $ad + da$, respectively; e_j is the residual effect assumed to be $N(0, \sigma_j^2)$; and

$$x_{ijr}^* = \begin{cases} 1 & \text{if genotype of QTL } r \text{ is } Q_r Q_r \\ -1 & \text{if genotype of QTL } r \text{ is } q_r q_r \end{cases};$$

$$z_{ijr}^* = \begin{cases} x_{ijr}^* & \text{if } j = 1 \\ -x_{ijr}^* & \text{if } j = 2 \end{cases};$$

$$\omega_{ijrs}^* = \begin{cases} 1/2 & \text{if the QTL genotype} \\ & \text{is } Q_r Q_r Q_s Q_s \text{ or } q_r q_r q_s q_s \\ -1/2 & \text{if the QTL genotype,} \\ & \text{is } Q_r Q_r q_s q_s \text{ or } q_r q_r Q_s Q_s \end{cases};$$

$$o_{ijrs}^* = \begin{cases} \omega_{ijrs}^* & \text{if } j = 1 \\ -\omega_{ijrs}^* & \text{if } j = 2 \end{cases}$$

$Q_r Q_r$ and/or $Q_s Q_s$ mean that the progeny is homozygous for the L3 allele, whereas $q_r q_r$ and/or $q_s q_s$ mean that the progeny is homozygous for the L22 allele.

Quantitative trait locus mapping was performed using the MIM model for design III with the software program Windows QTL Cartographer version 2.5 (Wang et al., 2012). Parameters for forward regression analysis were used on the basis of the increase in model likelihood. This procedure was repeated successively, adding QTLs on every round until no further QTLs were added, resulting in a model with r QTLs. As suggested by Garcia et al. (2008), the models with $r-1$ and r QTLs were compared based on Bayesian information criterion (BIC) (Schwarz, 1978), and the model with the lowest BIC value was selected. When no further decrease in BIC value was obtained by adding QTLs to the model, QTL positions were optimized. Akaike information criterion (AIC) (Akaike, 1974) was used to test the significance of two-way epistatic interactions between QTLs (Garcia et al., 2008) in the final model including all QTLs. Adjustment in QTL position was then performed. Confidence intervals were established as -1 LOD support interval (Lander and Botstein, 1989).

Table 1. Estimates of genetic variance between progeny ($\hat{\sigma}_g^2$), variance of interaction between progeny and parental inbred lines ($\hat{\sigma}_{gp}^2$), phenotypic variance on a progeny mean-basis ($\hat{\sigma}_p^2$), heritability coefficients on a mean-basis (\hat{h}^2), and backcross populations means (\bar{X}_j) for all phenotypic traits evaluated in maize (*Zea mays* L.) under low P availability.

| Parameters | PUE [†] | PAE [‡] | PUTIL [§] | GY [¶] | SDW [#] |
|---|------------------|------------------|--------------------|-------------------|-------------------|
| $\hat{\sigma}_g^2$ | 2139.19 | 0.007 | 929.39 | 263146 | 202062.58 |
| CI ^{††} | (1783–2636) | (0.006–0.008) | (789–1114) | (218,559–325,731) | (170,722–244,169) |
| $\hat{\sigma}_{gp}^2$ | 3926.90 | 0.012 | 1238.46 | 465599 | 303528.57 |
| CI | (3276–4821) | (0.009–0.014) | (1061–1471) | (388,227–575,102) | (257,817–363,354) |
| $\hat{\sigma}_p^2$ | 2799.55 | 0.01 | 1612.76 | 332663 | 317853.75 |
| CI | (2236–3609) | (0.007–0.013) | (1288–2079) | (265,648–428,818) | (253,827–409,728) |
| \hat{h}^2 | 0.76 | 0.66 | 0.58 | 0.79 | 0.64 |
| CI | (0.69–0.82) | (0.55–0.74) | (0.44–0.67) | (0.72–0.84) | (0.52–0.72) |
| $\bar{X}_{RILs \times L3}$ ^{‡‡} | 335.7 a | 0.74 a | 456.4 a | 3640.8 a | 3048.4 b |
| $\bar{X}_{RILs \times L22}$ ^{‡‡} | 261.6 b | 0.62 b | 421.1 b | 2853.3 b | 3327.0 a |

[†] PUE, phosphorus use efficiency.

[‡] PAE, phosphorus acquisition efficiency.

[§] PUTIL, phosphorus utilization internal efficiency.

[¶] GY, grain yield.

[#] SDW, shoot dry weight.

^{††} CI, confidence intervals at 95% probability are within parentheses for all parameters.

^{‡‡} Means followed with different letters, in each column, indicate a significant difference by F test ($p = 0.01$).

RESULTS

Genetic Variation for Phosphorus Use Efficiency Is Explained by Additive and Dominance Effects

For all P efficiency indexes (PUE, PAE, and PUTIL), as well as for GY and shoot dry weight (SDW), highly significant differences ($p < 0.01$) were detected in the combined analysis of variance for progeny and progeny by parent interactions (data not shown), indicating that both additive and dominance effects were important in explaining the genetic variation. Genetic variance ($\hat{\sigma}_g^2$) among RILs differed significantly from zero ($p \leq 0.05$) for all traits (Table 1), confirming the existence of genetic variability within the population. The averages of all traits except for SDW were significantly higher for progeny of backcrosses to L3 when compared with those from progeny of backcrosses to L22. These results indicate a significant effect of parental lines on the progeny. Heritability coefficients were high, especially for grain yield and PUE (0.79 and 0.76, respectively), whereas the estimates for PAE, PUTIL, and SDW ranged from 0.66 to 0.58 (Table 1).

Grain Yield Is Highly Correlated with PUE

A strong positive association was observed between PUE and GY, with estimates of phenotypic (\hat{r}_p) and genotypic (\hat{r}_G) correlations of 0.96 and 0.99, respectively (Table 2). Also, strong positive correlations were observed between PAE and GY and between PAE and PUE. On the other hand, PUTIL showed only moderate phenotypic correlation with both GY and PUE, although its genotypic correlation with PUE was strong (Table 2). Moderate correlations were also observed between GY and SDW. From the two

Table 2. Phenotypic (\hat{r}_p) and genotypic (\hat{r}_G) correlations among grain yield (GY), shoot dry weight (SDW), phosphorus use efficiency (PUE), and its components in maize (*Zea mays* L.) cultivated in low-P soil.

| Traits | PUE | | PAE [†] | | PUTIL [‡] | | SDW | |
|--------------------|-------------|-------------|------------------|-------------|--------------------|-------------|-------------|-------------|
| | \hat{r}_p | \hat{r}_G | \hat{r}_p | \hat{r}_G | \hat{r}_p | \hat{r}_G | \hat{r}_p | \hat{r}_G |
| GY | 0.96** | 0.99 | 0.85** | 0.95 | 0.56** | 0.74 | 0.42** | 0.51 |
| SDW | 0.38** | 0.43 | 0.53** | 0.54 | –0.10 | –0.10 | | |
| PUTIL [‡] | 0.60** | 0.81 | 0.17** | 0.54 | | | | |
| PAE [†] | 0.89** | 0.93 | | | | | | |

** Significant at the t test ($p = 0.01$); significance of genetic correlations were not tested.

[†] PAE, phosphorus acquisition efficiency.

[‡] PUTIL, phosphorus utilization internal efficiency.

components of PUE, only PAE was correlated with SDW, whereas PUTIL was not significantly correlated with SDW.

QTLs for Phosphorus Use Efficiency and Its Components

The genetic linkage map was constructed with 285 markers covering 1785.7 cM of the maize genome, with an average interval of 6.3 cM between adjacent markers (Fig. 1). Linkage groups ranged from 113.1 cM for chromosome 2 to 307.3 cM for chromosome 1.

Because of the strong correlation between GY and PUE, QTL analyses were performed only for P efficiency indexes in a low-P soil. Six QTLs were identified for PUE, six for PAE, and five for PUTIL (Fig. 1 and Table 3). Quantitative trait loci for PUE were mapped on chromosomes 1, 3, 4, 5, 7, and 8. Together, these QTLs explained 63.49% of the phenotypic variation for L3 backcross population (BC_{L3}) and 42.52% for L22 backcross population (BC_{L22}). Quantitative trait loci for PAE were highly coincident with those for PUE, explaining 60.30 and 38.18% of the

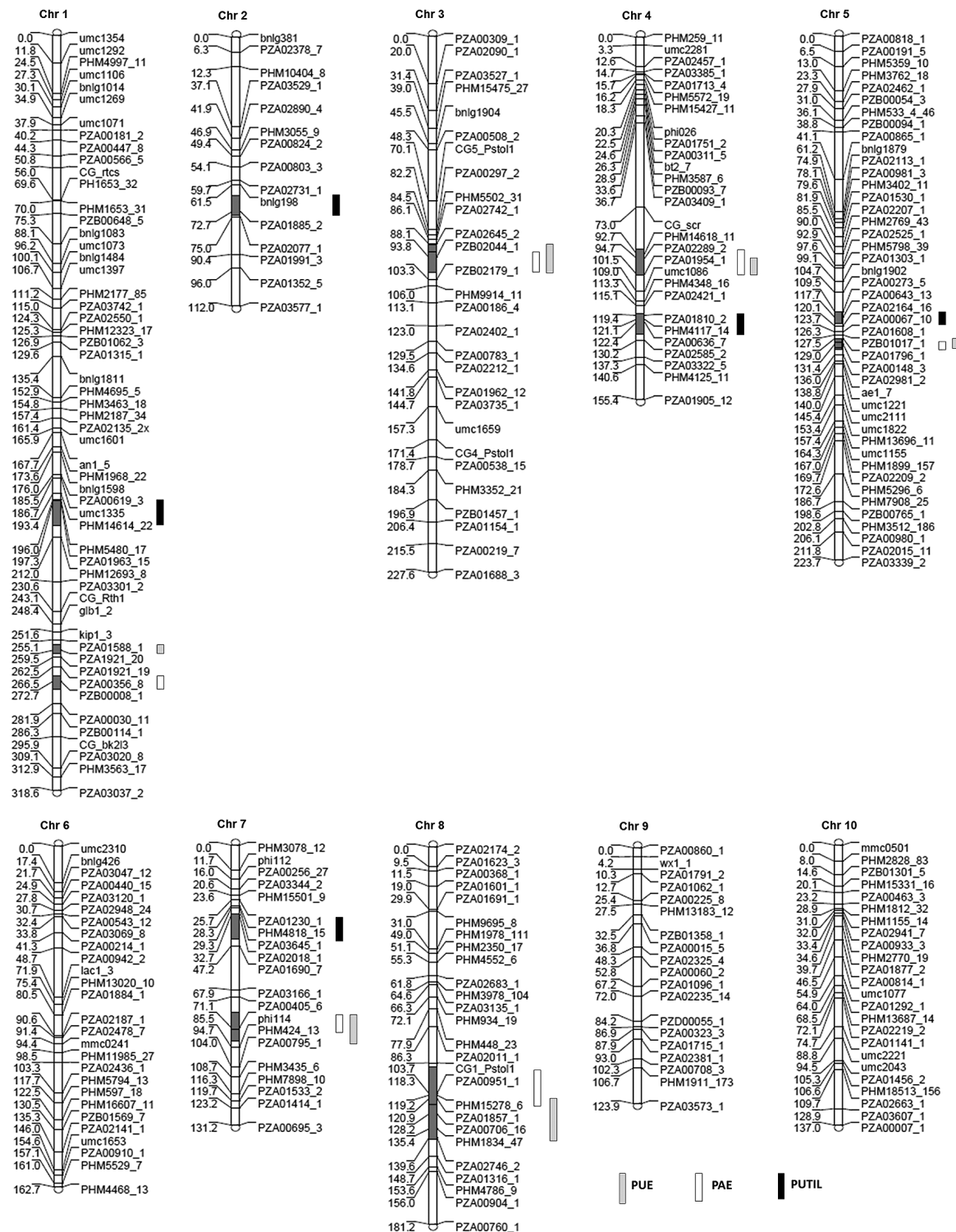


Figure 1. Genetic linkage map of quantitative trait loci (QTLs) for P efficiency indexes in maize (*Zea mays* L.), based on backcrosses of 140 recombinant inbred lines with both parental lines. For each chromosome (chr), marker names are indicated on the right and the genetic distance in centimorgans (cM) is on the left. The colored bars indicate the position of QTLs for PUE (phosphorus use efficiency), PAE (phosphorus acquisition efficiency), or PUTIL (phosphorus utilization internal efficiency), according to the legend. The bar length represents the confidence intervals based on one logarithm of odds drop-off.

total phenotypic variation for BC_{L3} and BC_{L22} , respectively (Table 4). Also, most QTLs for PUE and PAE showed augmented additive effects (a^*), with negative values, indicating that these QTLs were derived from L22, except for QTL on chromosome 4 for PUE ($qPUE4$) and for PAE ($qPAE4$).

Quantitative trait loci for PUTIL were mapped on chromosomes 1, 2, 4, 5, and 7. These QTLs explained 11.08 and 47.75% of the phenotypic variation for BC_{L3} and BC_{L22} , respectively. The QTLs for PUTIL were mapped at different positions from those for PUE and PAE. All

Table 3. Quantitative trait loci (QTLs) associated with P use efficiency (PUE) and its components (phosphorus acquisition efficiency [PAE] and phosphorus utilization internal efficiency [PUTIL]). Estimated QTL position and genetic effect, determination of the closest marker linked to QTL, logarithm of odds (LOD) score, fraction of the PUE and its components accounted for by each QTL [R_j^2 (%)].

| QTL | Chr [†] | Position (cM [‡]) | Marker | \hat{a}^* § | \hat{d}^* ¶ | LOD | R_1^2 (%) [#] | R_2^2 (%) ^{††} |
|---------|------------------|-----------------------------|-------------|---------------|---------------|------|--------------------------|---------------------------|
| qPUE1 | 1 | 258.7 | PZA01588_1 | -18.35 | 17.84 | 6.26 | 5.78 | 5.45 |
| qPUE3 | 3 | 92.8 | PZA02645_2 | -19.54 | 18.24 | 7.03 | 6.34 | 5.98 |
| qPUE4 | 4 | 97.4 | PZA02289_2 | 23.22 | 17.45 | 5.27 | 7.47 | 7.05 |
| qPUE5 | 5 | 130.1 | PZA00148_3 | -14.42 | 24.44 | 8.15 | 7.14 | 6.74 |
| qPUE7 | 7 | 83.4 | PZA00405_6 | -21.20 | 12.88 | 5.24 | 5.45 | 5.14 |
| qPUE8 | 8 | 127.3 | PZA01857_1 | -17.06 | 6.61 | 3.17 | 2.95 | 2.78 |
| qPAE1 | 1 | 272.4 | PZA00356_8 | -0.0465 | 0.0096 | 4.98 | 5.84 | 4.88 |
| qPAE3 | 3 | 94.7 | PZB02044_1 | -0.0285 | 0.0374 | 4.73 | 5.71 | 4.78 |
| qPAE4 | 4 | 97.4 | PZA02289_2 | 0.0293 | 0.0236 | 2.63 | 3.66 | 3.06 |
| qPAE5 | 5 | 131.1 | PZA01796_1 | -0.0267 | 0.0415 | 5.91 | 6.32 | 5.28 |
| qPAE7 | 7 | 81.4 | PZA00405_6 | -0.0584 | 0.0340 | 7.51 | 11.79 | 9.85 |
| qPAE8 | 8 | 121.3 | PZA01857_1 | -0.0245 | 0.0428 | 5.44 | 6.23 | 5.21 |
| qPUTIL1 | 1 | 200.9 | PZA01963_15 | 15.33 | 7.29 | 4.38 | 9.61 | 6.42 |
| qPUTIL2 | 2 | 71.5 | bnlg198 | 7.43 | 11.66 | 3.79 | 6.39 | 4.26 |
| qPUTIL4 | 4 | 123.0 | PZA00636_7 | 7.51 | 11.53 | 3.82 | 6.46 | 4.31 |
| qPUTIL5 | 5 | 120.3 | PZA02164_16 | 6.95 | 14.27 | 4.98 | 8.49 | 5.67 |
| qPUTIL7 | 7 | 37.4 | PZA02018_1 | 15.60 | 4.50 | 3.66 | 8.93 | 5.96 |

[†] Chr, chromosome.

[‡] cM, centimorgans.

[§] Augmented additive effect.

[¶] Augmented dominance effect.

[#] R_1^2 (%), fraction of the phenotypic variance explained by QTL effects in recombinant inbred lines (RILs) backcrossed to L3.

^{††} R_2^2 (%), fraction of the phenotypic variance explained by QTL effects in RILs backcrossed to L22.

Table 4. Summary of parameter estimation of the multiple interval mapping model for P use efficiency (PUE), P acquisition efficiency (PAE), and P internal utilization efficiency (PUTIL) in maize (*Zea mays* L.) RILs (recombinant inbred lines) backcrossed to parental lines L3 (BC_{L3}) or L22 (BC_{L22}).

| Parameters | BC _{L3} | | | BC _{L22} | | |
|--------------------------|------------------|-------|---------|-------------------|-------|---------|
| | PUE | PAE | PUTIL | PUE | PAE | PUTIL |
| $\hat{\sigma}_j^2$ † | 4111.33 | 0.02 | 2602.07 | 6864.67 | 0.02 | 2291.44 |
| $\hat{\sigma}_{P_j}^2$ ‡ | 11,260.40 | 0.05 | 2926.35 | 11,941.79 | 0.04 | 4385.23 |
| $\hat{\sigma}_{G_j}^2$ § | 7149.07 | 0.03 | 324.27 | 5077.12 | 0.01 | 2093.79 |
| R^2 (%) ¶ | 63.49 | 60.30 | 11.08 | 42.52 | 38.18 | 47.75 |

[†] $\hat{\sigma}_j^2$, residual variances for backcross *j*.

[‡] $\hat{\sigma}_{P_j}^2$, phenotypic variances for backcross *j*.

[§] $\hat{\sigma}_{G_j}^2$, genotypic variances for backcross *j*.

[¶] R^2 (%) = $\hat{\sigma}_a^2 / \hat{\sigma}_p^2$ is coefficient of determination.

QTLs for PUTIL were derived from the P-efficient line, L3. In general, all QTLs showed augmented dominance effect (d^*) with positive values, indicating that the dominance acted to increase P use efficiency and its components. The magnitude of d^* was similar to that of a^* for most QTLs, whereas the QTLs *qPUE8*, *qPAE1*, *qPUTIL1*, and *qPUTIL7* showed estimates of augmented dominance effect smaller than augmented additive effect, and only *qPUTIL5* presented d^* greater than a^* (Table 3).

Six epistatic interactions *aa + dd* and three *ad + da* were identified for PUE at low LOD values with prevalence of

positive signals, explaining individually only around 3% of the phenotypic variation (Table 5). The same number of epistatic interactions was observed for PAE, also explaining a small fraction of the phenotypic variation for both backcrossed populations. For PUTIL, only one epistatic interaction between *qPUTIL4* and *qPUTIL7* was detected, explaining approximately 1% of the phenotypic variation (Table 5).

DISCUSSION

To improve our understanding of the complex genetic architecture of PUE, this index was decomposed into PAE and PUTIL components, considering the total P content in the plant and the grain yield. All phenotypic traits showed significant genetic variability and high heritability, which are both important for QTL mapping. As expected, a high correlation was detected between GY and PUE, considering that PUE was calculated as the ratio between GY and the P_{soil}, which was a constant. Therefore, when soil fertility is uniform in the experimental area, GY is a valid estimate of PUE, avoiding extra costs for sampling and analyses of P content in the plant.

All QTLs for PUE were coincident or mapped close with the ones for PAE, which was expected given the strong correlation between PUE and PAE. Quantitative trait loci for PUE and PAE mapped on chromosome 1 (*qPUE1* and *qPAE1*) were flanked by root-related genes *rth1* and *bk213*, which were previously associated with

Table 5. Estimated epistatic effect, logarithm of odds (LOD) score, and fraction of the phenotypic variance accounted for by each individual epistatic interaction between quantitative trait loci (QTL) pairs [R_j^2 (%)]. PUE, phosphorus use efficiency; PAE, phosphorus acquisition efficiency; PUTIL, phosphorus utilization internal efficiency.

| QTL Pair | Effect [†] | | LOD | R_1^2 (%) [‡] | R_2^2 (%) [§] |
|------------------|---------------------|----------------|------|--------------------------|--------------------------|
| | <i>aa + dd</i> | <i>ad + da</i> | | | |
| qPUE1, qPUE3 | 53.65 | | 1.23 | 1.56 | 1.47 |
| qPUE1, qPUE5 | -54.73 | | 1.39 | 1.66 | 1.56 |
| qPUE1, qPUE5 | 55.81 | | 1.55 | 1.71 | 1.62 |
| qPUE3, qPUE5 | 39.21 | | 0.75 | 0.85 | 0.80 |
| qPUE3, qPUE7 | 47.18 | | 0.84 | 1.23 | 1.16 |
| qPUE4, qPUE8 | -35.53 | | 0.55 | 0.69 | 0.65 |
| qPUE3, qPUE7 | | 38.75 | 0.71 | 0.83 | 0.78 |
| qPUE3, qPUE8 | | 37.58 | 0.78 | 0.78 | 0.74 |
| qPUE5, qPUE7 | | 81.45 | 3.14 | 3.64 | 3.44 |
| qPAE1, qPAE4 | -0.033 | | 0.48 | 0.57 | 0.68 |
| qPAE1, qPAE5 | 0.053 | | 1.50 | 1.48 | 1.77 |
| qPAE3, qPAE5 | 0.044 | | 1.03 | 1.05 | 1.26 |
| qPAE3, qPAE7 | 0.058 | | 1.37 | 1.81 | 2.17 |
| qPAE4, qPAE5 | 0.037 | | 0.57 | 0.74 | 0.88 |
| qPAE5, qPAE7 | 0.036 | | 0.63 | 0.70 | 0.84 |
| qPAE3, qPAE7 | | 0.047 | 1.16 | 1.18 | 1.42 |
| qPAE3, qPAE8 | | 0.044 | 1.08 | 1.03 | 1.24 |
| qPAE5, qPAE7 | | 0.068 | 2.44 | 2.46 | 2.95 |
| qPUTIL4, qPUTIL7 | 11.93 | | 0.46 | 0.80 | 1.20 |

[†] Epistatic effects in kg kg⁻¹.

[‡] R_1^2 (%), fraction of the phenotypic variance explained by epistatic effects in RILs backcrossed to L3.

[§] R_2^2 (%), fraction of the phenotypic variance explained by epistatic effects in RILs backcrossed to L22.

lateral root length (Brady et al., 2006) and with root hair elongation (Wen et al., 2005), respectively (Fig. 1). Additionally, this genomic region is known to harbor QTLs for root development under low-P conditions (Zhu et al., 2005; Chen et al., 2008, 2009). As P has limited mobility in the soil, improving root development is expected to enhance P acquisition, which provides a partial explanation for the co-localization of QTLs for PUE and PAE with candidate genes controlling root morphology traits. Furthermore, the parental lines used here differed in early root traits (Brasil et al., 2007; de Sousa et al., 2012).

Most QTLs for PUTIL were not coincident with those for PUE and PAE, reflecting a possible complementation of P internal utilization and P acquisition mechanisms for PUE. This result is also in agreement with the low phenotypic correlation between PUTIL and PAE. Indeed, both indexes are controlled by different mechanisms. Phosphorus acquisition efficiency is related to modifications in root architecture and morphology, organic acids exudation (Lynch and Brown, 2001), and association with mycorrhizae (Bucher, 2007). However, the mechanisms controlling PUTIL comprise the P transport within the plant, the remobilization and distribution of internal P, which are important to maintain the plant metabolism in low P concentrations (Parentoni et al., 2012).

All QTLs for PUE, PAE, and PUTIL showed positive augmented dominance effects, and most of them with magnitude similar to that of their corresponding additive effects, indicating a complete dominance for these QTLs. This suggests that dominance of favorable alleles can be the cause of heterosis for these traits, in agreement with published data using generation means (Parentoni and Souza Júnior, 2008; Parentoni et al., 2010). Moreover, our estimates of additive and dominance variances can be considered more accurate once we used a RIL population, which dissipated linkage disequilibrium across generations of selfing, reducing pseudo-overdominance effects, as proposed by Comstock and Robinson (1952).

Most QTLs for increasing PUE and PAE were derived from the P-inefficient line, L22. This result was unexpected, since the other parental line, L3, was previously characterized as P-efficient, and the RIL population backcrossed with L3 presented superior phenotypic means for both traits. Interestingly, the genetic variance and the proportion of phenotypic variance explained by QTLs for PUE and PAE were higher for the RIL population backcrossed with L3 (Table 4), a population where the alleles donated by L3 were mainly in homozygosity. Thus, this backcross may have favored the expression of dominant alleles from L22, which were in heterozygosity with those donated by L3, leading to the expression of dominance effects. A similar situation was observed for PUTIL, a trait with all favorable QTLs derived from L3. For this index, the population backcrossed with L22 showed superior genetic variance and proportion of phenotypic variance explained by QTLs (Table 4). This hypothesis is supported by the prevalence of dominance effects, which were detected in a large proportion of QTLs mapped for all P efficiency indexes.

With the advances in methods to dissect the genetic architecture of complex traits, identifying epistatic interactions between QTLs has received more attention (Garcia et al., 2008; Schön et al., 2010; Ku et al., 2012). Different epistatic interactions were detected for P efficiency indexes, providing an additional level of complexity to the genetic inheritance of these traits. Our estimates of epistasis showed predominance of positive values explaining small fractions of the phenotypic variation. Garcia et al. (2008) demonstrated that only negative, additive by additive (*aa*) epistasis increases heterosis in a similar manner to dominance effects. If *aa* is important for heterosis and most of its effects are negative, values of *aa + dd* estimates would be predominantly negative, because when *dd* is positive the effects tend to cancel each other and are difficult to detect. Our results suggest that, although *aa* epistasis might be present in our analysis, it is unlikely to contribute to heterosis for P efficiency in maize because of their signs.

Finally, our work makes an important contribution to designing breeding strategies for improving PUE in maize, since our QTL analysis of PUE indexes was based

on grain yield. In contrast, most published QTLs associated with PUE in maize were based on measurements of shoot dry weight in plants at their initial stages of development (Chen et al., 2008, 2009, 2011; Maia et al., 2011). These studies do not address the genetic relationship between early PUE indexes and grain yield as examined in our work. Furthermore, we provide information on the genetic control of PUE in maize cultivated in tropical conditions, which was not explored in other studies. As QTLs for PAE and PUTIL were mapped in different genomic regions, both QTLs can be used as targets for marker-assisted selection to pyramid complementary mechanisms of P use efficiency.

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References

- Akaike, H. 1974. A new look at the statistical model identification. *IEEE Trans. Autom. Control* 19:716–723. doi:10.1109/TAC.1974.1100705
- Brady, S.M., S. Song, K.S. Dhugga, A. Rafalski, and P.N. Benfey. 2006. Combining expression and comparative evolutionary analysis: The COBRA gene family. *Plant Physiol.* 143:172–187. doi:10.1104/pp.106.087262
- Brasil, E.C., V.M.C. Alves, I.E. Marriel, G.V.E. Pitta, and J.G. de Carvalho. 2007. Dry matter and mineral nutrition in efficient corn genotypes in relation to phosphorus acquisition. (Portuguese, with English abstract). *Ciênc. Agrotec.* 31:704–712. doi:10.1590/S1413-70542007000300016
- Bucher, M. 2007. Functional biology of plant phosphate uptake at root and mycorrhiza interface. *New Phytol.* 173:11–26. doi:10.1111/j.1469-8137.2006.01935.x
- Chen, J., Y. Cai, L. Xu, J. Wang, W. Zhang, G. Wang, D. Xu, T. Chen, X. Lu, H. Sun, A. Huang, Y. Liang, G. Dai, H. Qin, Z. Huang, Z. Zhu, Z. Yang, J. Xu, and S. Kuang. 2011. Identification of QTLs for biomass production in maize (*Zea mays* L.) under different phosphorus levels at two sites. *Front. Agric. China* 5:152–161. doi:10.1007/s11703-011-1077-3
- Chen, J., L. Xu, Y. Cai, and J. Xu. 2008. QTL mapping of phosphorus efficiency and relative biologic characteristics in maize (*Zea mays* L.) at two sites. *Plant Soil* 313:251–266. doi:10.1007/s11104-008-9698-x
- Chen, J., L. Xu, Y. Cai, and J. Xu. 2009. Identification of QTLs for phosphorus utilization efficiency in maize (*Zea mays* L.) across P levels. *Euphytica* 167:245–252. doi:10.1007/s10681-009-9883-x
- Collins, N.C., F. Tardieu, and R. Tuberosa. 2008. Quantitative trait loci and crop performance under abiotic stress: Where do we stand? *Plant Physiol.* 147:469–486. doi:10.1104/pp.108.118117
- Comstock, R.E., and H.F. Robinson. 1952. Estimation of average dominance of genes. In: J.W. Gowen, editor, *Heterosis*. Iowa State College Press, Ames. p. 494–516.
- Cordell, D., J.O. Drangert, and S. White. 2009. The story of phosphorus: Global food security and food for thought. *Glob. Environ. Change* 19:292–305. doi:10.1016/j.gloenvcha.2008.10.009
- Creste, S., A.T. Neto, and A. Figueira. 2001. Detection of single sequence repeat polymorphisms in denaturing polyacrylamide sequencing gels by silver staining. *Plant Mol. Biol. Rep.* 19:299–306. doi:10.1007/BF02772828
- Cruz, C.D. 2013. GENES: A software package for analysis in experimental statistics and quantitative genetics. *Acta Sci.* 35:271–276.
- de Sousa, S.M., T. Randy, R.T. Clark, F.F. Mendes, A.C. Oliveira, M.J.V. Vasconcelos, S.N. Parentoni, L.V. Kochian, C.T. Guimarães, and J.V. Magalhães. 2012. A role for root morphology and related candidate genes in P acquisition efficiency in maize. *Funct. Plant Biol.* 39:925–935. doi:10.1071/FP12022
- Fageria, N.K. 1998. Optimizing nutrient use efficiency in crop production. (Portuguese, with English abstract). *Rev. Bras. Eng. Agríc. Ambient.* 2:6–16.
- FAO. 2010. How to feed the world in 2050. Rome. www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf (accessed 18 Apr. 2014).
- FAOSTAT. 2013. Statistical database of the Food and Agriculture Organization of the United Nations. FAO, Rome, Italy. <http://faostat.fao.org> (accessed 4 June 2013).
- Gamuyao, R., J.H. Chin, J. Pariasca-Tanaka, P. Pesaresi, S. Catausan, C. Dalid, I. Slamet-Loedin, E.M. Tecson-Mendoza, M. Wissuwa, and S. Heuer. 2012. The protein kinase Pst1l from traditional rice confers tolerance of phosphorus deficiency. *Nature* 488:535–539. doi:10.1038/nature11346
- Garcia, A.A.F., S. Wang, A.E. Melchinger, and Z.B. Zeng. 2008. Quantitative trait loci mapping and the genetic basis of heterosis in maize and rice. *Genetics* 180:1707–1724. doi:10.1534/genetics.107.082867
- Hallauer, A.R., M.J. Carena, and J.B. Miranda Filho. 2010. *Quantitative genetics in maize breeding*. Springer, New York.
- Hochholdinger, F., T.J. Wen, R. Zimmermann, P. Chimot-Marolle, O. da Costa e Silva, W. Bruce, K.R. Lamkey, U. Wienand, and P.S. Schnable. 2008. The maize (*Zea mays* L.) *roothairless3* gene encodes a putative GPI-anchored, monocot-specific, COBRA-like protein that significantly affects grain yield. *Plant J.* 54:888–898. doi:10.1111/j.1365-313X.2008.03459.x
- Kosambi, D.D. 1944. The estimation of map distances from recombination values. *Ann. Eugen.* 12:172–175. doi:10.1111/j.1469-1809.1943.tb02321.x
- Ku, L.X., Z.H. Sun, C.L. Wang, J. Zhang, R.F. Zhao, H.Y. Liu, G.Q. Tai, and Y.H. Chen. 2012. QTL mapping and epistasis analysis of brace root traits in maize. *Mol. Breed.* 30:697–708. doi:10.1007/s11032-011-9655-x
- Lander, E.S., and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185–199.
- Li, M., X. Guo, M. Zhang, X. Wang, G. Zhang, Y. Tian, and Z. Wang. 2010. Mapping QTLs for grain yield and yield components under high and low phosphorus treatments in maize (*Zea mays* L.). *Plant Sci.* 178:454–462. doi:10.1016/j.plantsci.2010.02.019
- Lim, J., J.W. Jung, C.E. Lim, M.H. Lee, B.J. Kim, M. Kim, W.B. Bruce, and P.N. Benfey. 2005. Conservation and diversification of SCARECROW in maize. *Plant Mol. Biol.* 59:619–630. doi:10.1007/s11103-005-0578-y
- Lincoln, S.E., M.J. Daly, and E.S. Lander. 1992. *Constructing genetic maps with Mapmaker Exp 3.0*. 3rd ed. Whitehead Institute for Biometrical Research, Cambridge, MA.

- Lynch, J.P. 2011. Root phenes for enhanced soil exploration and phosphorus acquisition: Tools for future crops. *Plant Physiol.* 156:1041–1049. doi:10.1104/pp.111.175414
- Lynch, J.P., and K.M. Brown. 2001. Topsoil foraging—An architectural adaptation of plants to low phosphorus. *Plant Soil* 237:225–237. doi:10.1023/A:1013324727040
- Maia, C., J.C. do Vale, R. Fritsche-Neto, P.C. Cavatte, and G.V. Miranda. 2011. Difference between breeding for nutrient use efficiency and nutrient stress tolerance. *Crop Breed. Appl. Biotechnol.* 11:270–275. doi:10.1590/S1984-70332011000300010
- Melchinger, A.E., H.P. Piepho, H.F. Utz, J. Muminović, T. Wegenast, O. Törjék, T. Altmann, and B. Kust. 2007. Genetic basis of heterosis for growth-related traits in arabidopsis investigated by testcross progenies of near-isogenic lines reveals a significant role of epistasis. *Genetics* 117:1815–1825. doi:10.1534/genetics.107.077537
- Moll, R.H., E.J. Kamprath, and W.A. Jackson. 1981. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. *Agron. J.* 74:562–564. doi:10.2134/agronj1982.00021962007400030037x
- Ninamango-Cárdenas, F.E., C.T. Guimarães, P.R. Martins, S.N. Parentoni, N.P. Carneiro, and E. Paiva. 2003. Mapping QTLs for aluminum tolerance in maize. *Euphytica* 130:223–232. doi:10.1023/A:1022867416513
- Parentoni, S.N., F.F. Mendes, and L.J.M. Guimarães. 2012. Breeding for phosphorus use efficiency. In: R. Fritsche-Neto and A. Borém, editors, *Plant breeding for abiotic stress tolerance*. Springer, New York. p. 67–86.
- Parentoni, S.N., and C.L. Souza Júnior. 2008. Phosphorus acquisition and internal utilization efficiency in tropical maize genotypes. *Pesqui. Agropecu. Bras.* 43:893–901.
- Parentoni, S.N., C.L. Souza Junior, V.M.C. Alves, E.E.G. Gama, A.M. Coelho, A.C. de Oliveira, C.T. Guimaraes, M.J.V. Vasconcelos, C.A.P. Pacheco, W.F. Meirelles, J.V. Magalhaes, L.J.M. Guimarães, A.R. da Silva, F.F. Mendes, and R.E. Schaffert. 2010. Inheritance and breeding strategies for phosphorus efficiency in tropical maize (*Zea mays* L.). *Maydica* 55:1–15.
- Saghai-Maroo, M.A., K.M. Soliman, R.A. Jorgensen, and R.W. Allard. 1984. Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci. USA* 81:8014–8018. doi:10.1073/pnas.81.24.8014
- Schön, C.C., B.S. Dhillon, H.F. Utz, and A.E. Melchinger. 2010. High congruency of QTL positions for heterosis of grain yield in three crosses of maize. *Theor. Appl. Genet.* 120:321–332. doi:10.1007/s00122-009-1209-9
- Schwarz, G. 1978. Estimating the dimension of a model. *Ann. Stat.* 6:461–464. doi:10.1214/aos/1176344136
- SAS Institute. 1999. SAS STAT 9.2 user's guide. Vol. 6. SAS Institute, Cary, NC.
- Taramino, G., M. Sauer, J.L. Stauffer, D. Multani, X. Niu, H. Sakai, and F. Hochholdinger. 2007. The maize (*Zea mays* L.) *RTCS* gene encodes a LOB domain protein that is a key regulator of embryonic seminal and post-embryonic shoot-borne root initiation. *Plant J.* 50:649–659. doi:10.1111/j.1365-313X.2007.03075.x
- Vance, C.P., C. Uhde-Stone, and D.L. Allan. 2003. Phosphorus acquisition and use: Critical adaptations by plants for securing a nonrenewable resource. *New Phytol.* 157:423–447. doi:10.1046/j.1469-8137.2003.00695.x
- Wang, S., C.J. Basten, and Z.B. Zeng. 2012. *Windows QTL Cartographer 2.5*. Department of Statistics, North Carolina State University, Raleigh.
- Wen, T.J., F. Hochholdinger, M. Sauer, W. Bruce, and P.S. Schnable. 2005. The *roothairless1* gene of maize encodes a homolog of *sec3*, which is involved in polar exocytosis. *Plant Physiol.* 138:1637–1643. doi:10.1104/pp.105.062174
- Zhu, J., S.M. Kaepler, and J.P. Lynch. 2005. Mapping of QTL controlling root hair length in maize (*Zea mays* L.) under phosphorus deficiency. *Plant Soil* 270:299–310. doi:10.1007/s11104-004-1697-y