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ORIGINAL PAPER

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Detection of quantitative trait loci for seminal root traits in maize (*Zea mays* L.) seedlings grown under differential phosphorus levels

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Abstract Suboptimal phosphorus availability is a primary constraint for terrestrial plant growth. Seminal roots play an important role in acquisition of nutrients by plant seedlings. The length and number of seminal roots may be particularly important in acquisition of immobile nutrients such as phosphorus by increasing soil exploration. The objective of this study was to identify quantitative trait loci (QTL) controlling seminal root growth in response to phosphorus stress in maize, and to characterize epistatic interactions among QTL. Seminal root length and number were evaluated in 162 recombinant inbred lines derived from a cross between B73 and Mo17 in seedlings grown in a controlled environment. B73 and Mo17 significantly differed for seminal root length under low phosphorus, but not under adequate phosphorus conditions. Seminal root length of the population grown under low phosphorus ranged from 0 to 79.2 cm with a mean of 32.3 cm; while seminal root length of plants grown under high phosphorus ranged from 0.67 to 59.0 cm with a mean of 23.4 cm. Under low phosphorus, one main-effect QTL was associated with seminal root length and three QTL with seminal root number; under high phosphorus, two QTL with seminal root length and three QTL for seminal root number. These accounted for 11, 25.4, 22.8, and 24.1% of the phenotypic variations for seminal root length and number at low phosphorus, and seminal root length and number at high phosphorus, respectively. Di-genic epistatic loci were detected for seminal root length at low phosphorus (two pairs) seminal root number at low phosphorus (eight pairs), seminal root length at high phosphorus (four pairs), and seminal root number at high phosphorus (two pairs), which accounted for 23.2, 50.6, 32.2, and 20.3% of the total variations, respectively. Seminal root traits observed here were positively yet weakly correlated with shoot biomass in the field under low phosphorus, although no coincident QTL were detected. These results suggest that epistatic interactions are important in controlling genotypic variation associated with seedling seminal root traits.

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Introduction

Low soil phosphorus availability is a primary constraint to plant growth in terrestrial ecosystems (Lynch and Deikman 1998). Maize (*Zea mays* L.) is generally considered to have a high fertility requirement, but variation has been known to exist among maize genotypes for phosphorus efficiency for more than a century (Anonymous 1887; Naismith et al. 1974; Clark and Brown 1974; Nielsen and Barber 1978; Da Silva and Gabelman 1993; Kaeppler et al. 2000; Gaume et al. 2001; Fan et al. 2003; Zhu and Lynch 2004; Zhu et al. 2005a). A better understanding of the genetic basis of root traits that improve phosphorus acquisition would facilitate the development of phosphorus efficient maize genotypes, which would be beneficial in low-input agroecosystems and would improve the sustainability of high-input agroecosystems (Lynch 1998).

The root system of 2-week-old maize seedlings consists of the primary root, lateral root branches originating from the primary root, and seminal roots (Zhu 2003; Zhu et al. 2005b, c). For the first 2–3 weeks after germination, the seminal roots comprise the bulk of the seedling root system (Feldman 1994). Seminal root primordia are already present in the ungerminated mature maize kernel, with the embryo typically containing from 3 to 7 seminal root primordia (Feldman 1994). Seminal roots play a key role in the acquisition of immobile nutrients such as phosphorus by determining the spatial and

temporal domains of soil foraging as well as inter-root competition.

Recent advances in the availability of well-developed molecular genetic maps (Senior et al. 1996) and the definition of distinct traits determining root architecture (e.g., Lynch and Brown 2001) make it possible to identify and locate genes or quantitative trait loci (QTL) for root traits. Main-effect QTL affecting a wide range of root traits of maize have previously been reported for root growth at low phosphorus and responsiveness to arbuscular mycorrhizal fungi (Kaeppler et al. 2000), drought tolerance (Tuberosa et al. 2002, 2003), and root hair length and lateral rooting traits conferring phosphorus efficiency (Zhu 2003; Zhu and Lynch 2004; Zhu et al. 2005b, c). The QTL analysis of lateral rooting and root hair traits demonstrates that these complex root traits are genetically controlled under high and low phosphorus availability by many genes or QTL and/or loci with epistatic effects (Zhu et al. 2005b, c).

Studies have shown that epistasis is important for specific combinations of maize inbred lines (Jinks 1955; Bauman 1959, Darrah and Hallauer 1972; Lamkey et al. 1995; Wolf and Hallauer 1997). Other studies, however, have demonstrated that epistasis is a non-significant component of genetic variability in maize populations (Eberhart et al. 1966; Chi et al. 1969; Silva and Hallauer 1975; Hinze and Lamkey 2003). The QTL analysis has typically uncovered little evidence for epistasis (Edwards et al. 1987; Xiao et al. 1995). Recently, two maize traits, plant and inflorescence architecture, were found to interact epistatically (Doebley et al. 1995). McMullen et al. (2001) found the biological basis of epistasis between QTL for flavone and 3-deoxyanthocyanin synthesis in maize. Determining the contribution of epistasis to complex traits is important for understanding the genetic basis of complex traits. No information is available on the role of epistasis in controlling seminal root traits in maize.

The objectives of this research were to (1) identify QTL for seminal root length and number in response to phosphorus stress, and (2) determine whether epistasis is important in explaining genetic variation for seminal root traits. We report here the results of a genetic mapping analysis of main effects and di-genic interaction of QTL controlling seminal root traits in maize grown at low and adequate phosphorus. This research contributes towards a long-term goal of developing genetic tools that will facilitate breeding more phosphorus efficient maize genotypes.

Materials and methods

Growth chamber study

Mapping population

Recombinant inbred lines (RILs) from the cross B73×Mo17 were increased at the University of Wisconsin, Madison, USA, and were originally supplied by Charles Stuber and Lynn Senior at North Carolina State University. 'Mo17' is a phosphorus efficient genotype, and 'B73' is a phosphorus inefficient genotype (Kaeppler et al. 2000). A total of 162 RILs were analyzed in this study. The RIL population was previously genotyped with 196 RFLP, SSR, and isozyme markers (Senior et al. 1996). This genetic linkage map has 27 markers on chromosome 1, 14 on chromosome 2, 15 on chromosome 3, 23 on chromosome 4, 32 on chromosome 5, 21 on chromosome 6, 13 on chromosome 7, 17 on chromosome 8, 18 on chromosome 9, and 16 on chromosome 10. The map covers 1,703 cM with an average interval of 8.73 cM.

Experimental design

The experiment was a randomized complete block design with a split-plot arrangement of treatments. The main plots were low- and high phosphorus; subplots were 164 genotypes (2 parents plus 162 lines). There were three replicates staggered in time. For each replicate, there were four plants.

Cigar roll culture system

Maize seeds of two parents and their 162 F_{10}-derived RILs were surface sterilized for 1 min in 0.5% solution of NaOCl and then washed in deionized H_2O before germination. In each replicate, four typical seeds from the seed stock for a genotype were selected and wrapped in brown germination paper (Anchor Paper, St. Paul, MN, USA) as a cigar roll. Two batches of 164 cigar rolls were soaked vertically in two plastic containers (Sterilite, Townsend, MA, USA) with $47.5\times35\times25$ cm^3 (length × width × height) filled with 5 l nutrient solution with low or high phosphorus. The low phosphorus nutrient solution consisted of (in μM): K (3,000), NO_3 (7,000), NH_4 (1,000), Ca (2,000), SO_4 (500), Mg (500), Cl (25), B (12.5), Mn (1), Zn (1), Cu (0.25) Mo (0.25) and EDTA-Fe (25), which had 0.2 μM phosphorus. For high phosphorus solution, 0.5% (W/V) of alumina buffered media was added to the low phosphorus nutrient solution, which continuously maintained 62 μM phosphorus in solution (Lynch et al. 1990). Seedlings were germinated in darkness at 28±1°C in a growth chamber for 3 days, then grown under a photoperiod of 14/10 h at 28/24°C (light/darkness) with photosynthetically active radiation (PAR) of 200 μmol photons m^{-2} s^{-1} at the soil level. The relative humidity was 65%. The nutrient solution pH was adjusted to 6.0 daily.

Measurement of seminal root length and number

Plant seedlings were harvested 14 days after germination and the roots were preserved in 30% ethanol immediately. At the time of harvest, the root system consisted of a primary root with emerged lateral roots, and 0–8 seminal roots. Seminal roots were dissected from seedlings and scanned using image analysis software

(WinRhizo Pro, Régent Instruments, Québec, Canada). Seminal root length was determined by the software, and root number was counted (Zhu and Lynch 2004; Zhu et al. 2005c).

Data analysis

Mean coefficient of variation was 20.2% for seminal root length at low phosphorus, 22.6% for seminal root number at low phosphorus, 22.9% for seminal root length at high phosphorus, and 23.1% for seminal root number at high phosphorus. The data from the inbred screening experiment were first subjected to ANOVA using a general linear model that included phosphorus and genotype as factorial treatments (Minitab Inc. University Park, PA, USA). Genotype and phosphorus levels were considered fixed effects, and replicates were random. There was no significant difference among replicates for seminal root length, while there was a significant difference among replicates for seminal root number in the cigar roll experiment ($P<0.01$). There was no significant difference for seminal root length among replicates at both low and high phosphorus, and no significant difference for seminal root number among replicates at high phosphorus; while there was significant difference for seminal root number among replicates at low phosphorus ($P<0.01$). The phosphorus level × genotype interaction was highly significant (data not shown) for both seminal root length and seminal root number. Significance of genotypes within phosphorus level was determined by separate ANOVA within a phosphorus level. The genotypic differences were significant for both seminal root length and number at low or high phosphorus level. To identify transgressive segregation, a paired *t* test was performed for each inbred line relative to the most similar parent under different phosphorus treatments (Minitab Inc., University Park, USA).

Arithmetic mean values across three replicates were calculated for each variable for RILs and used for QTL mapping. Main-effect QTL and epistasis in the means of parameters were analyzed using the program QTL Mapper (Wang et al. 1999). This program is based on the method of mixed linear models (Zhu 1998; Zhu and Weir 1998). The significance level of the genome scan was set at 0.05, and the pointwise significance level of QTL mapping was 0.000066. Thus, the average LOD of 2.0 was used for claiming main-effect QTL in this study.

Correlation analysis was performed for shoot dry weight and seminal root length and number at low phosphorus in the RIL population (Minitab Inc., University Park, PA). Multiple linear regression was conducted for shoot dry weight using seed phosphorus content, root hair length, and seminal root length and number at low phosphorus as variants in the RIL population (Minitab Inc., University Park, PA). The data of seed phosphorus content and root hair length were from Zhu et al. (2005c).

Field study

Maize seeds of the two parents and their 162 S_5-derived RILs were planted in a randomized complete block design with single row plots at the Marshfield, WI Agricultural Research Station in 1998, 1999, and 2000 and at the Arlington, WI Agricultural Research Station in 1999 and 2000. For the low P treatment, the soil of the Marshfield plot was characterized as a Withee silt loam (fine-loamy, mixed, frigid: Typic Ochraqualf), which had a Bray P1 test of 17 ppm P; the soil at the Arlington site was characterized as Plano silt loam (fine-silty, mixed, mesic; Typic Argiudoll), which had a Bray P1 test of 11 ppm P. Planting density in all environments was 69,136 plants ha^{-1}. Soil pH was 6.4 and organic matter 2.7% at Marshfield, and soil pH was 5.8 and organic matter 3.6% at Arlington. Potassium levels were 83 ppm at Marshfield and 75 ppm at Arlington with no K deficiency symptoms observed. Shoot weights were taken approximately 6 weeks after planting in the five field environments. Five shoots per replicate were harvested and dried for one week in forced air dryers before being weighed. Mean values of shoot dry weight were calculated across five replicates for the parents and their RILs, since genotype by environment interactions were not significant ($P\leq0.05$) (data not shown).

Results

Phenotypic variation

The phenotypic values of the parents and of the RI population are presented in Figs. 1 and 2. Low phosphorus availability increased seminal root length in Mo17, the phosphorus efficient parent, but significantly decreased seminal root length in B73, the phosphorus inefficient parent ($P<0.01$) (Fig. 1). Seminal root number of Mo17 was stimulated by low phosphorus availability with an increase of 43%, while seminal root number of B73 was inhibited by low phosphorus ($P<0.01$) (Fig. 2). Under high phosphorus availability, there was no significant difference for both seminal root length and number between the two parents (Figs. 1, 2). Transgressive segregation for seminal root length and number in the RI population were observed. Seminal root length of the population under low phosphorus ranged from 0 to 79.2 cm with a mean of 32.3 cm; while under high phosphorus culture, it ranged between 0.67 and 59.0 cm with a mean of 23.4 cm (Fig. 1.). Seminal root number of the population under low phosphorus ranged from 0 to 8 with a mean of 2.76; while under high phosphorus culture, it ranged between 0.33 and 5.67 with a mean of 2.59 (Fig. 2).

Main-effect QTL analysis

QTL were detected for four traits including seminal root length and number at low phosphorus, and seminal root

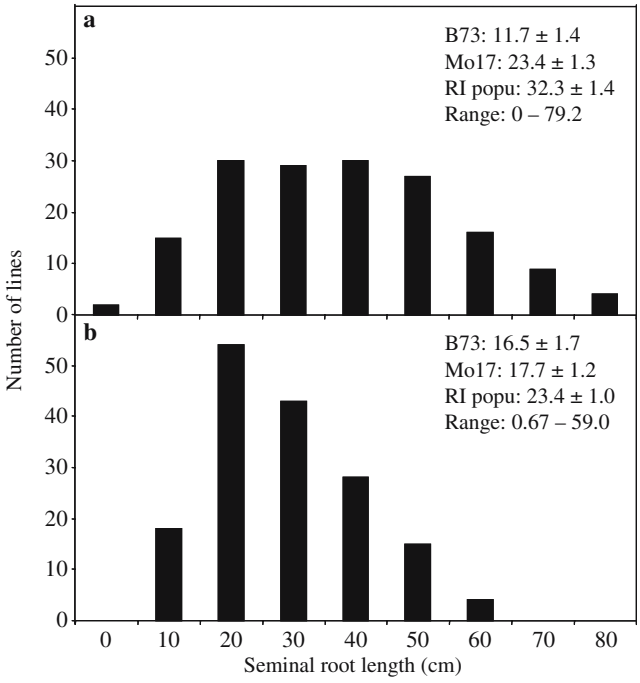

Fig. 1 Frequency distribution of seminal root length in 162 maize RILs from B73×Mo17 under low phosphorus (**a**), and high phosphorus (**b**). Data are means±SE ($n=3$)

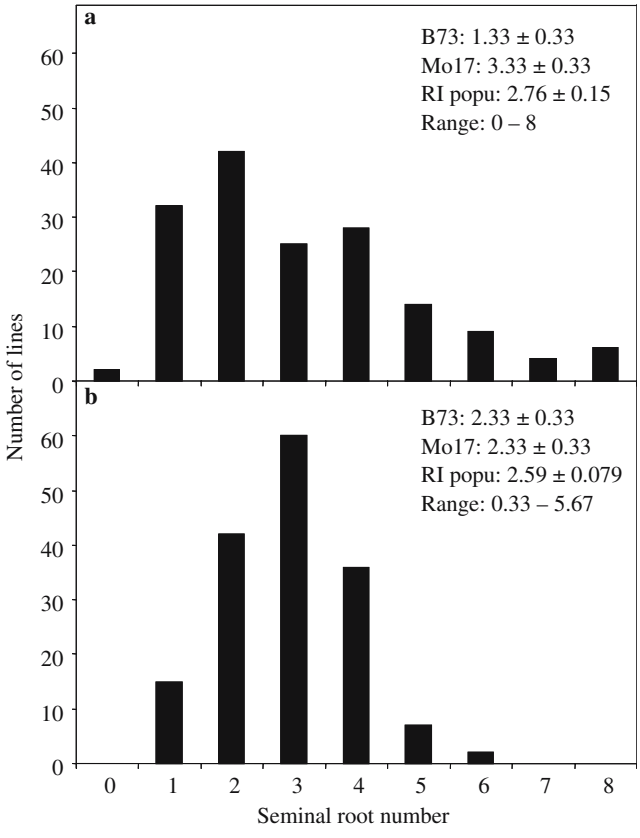

Fig. 2 Frequency distribution of seminal root number in 162 maize RILs from B73×Mo17 under low phosphorus (**a**), and high phosphorus (**b**). Data are means±SE ($n=3$)

Table 1 List of detected QTL for seminal root length (SRL) and seminal root number (SRN) under low and high phosphorus conditions[a]

Traits	Chromosome	Marker interval	LOD	R^2	Additive[b]
At low phosphorus availability					
SRL	2	umc34/bn112.09	5.55	11.0	5.42
SRN	1	csu3/umc133b	2.33	7.3	−0.35
	2	umc34/bn112.09	3.90	6.8	0.53
	6	phi126/rxo1	6.73	11.3	0.65
At high phosphorus availability					
SRL	1	amp1/umc128	3.58	10.9	−3.34
	2	bn112.09/umc131	6.08	11.9	4.51
SRN	1	bn17.08b/amp1	2.67	7.5	−0.27
	2	bn112.09/umc131	3.67	7.0	0.30
	3	bn16.16/umc17	2.01	9.6	0.22

LOD $\log_{10}$-likelihood
[a] Threshold probability <0.005
[b] Positive values indicate that Mo17 carries the allele for an increase in the trait, and negative values indicate that B73 contributes the allele for an increase in the trait

length and number at high phosphorus (Table 1). Three QTL flanked by csu3/umc133b, umc34/bn112.09, and phi126/rxo1 on chromosomes 1, 2, and 6, respectively, were identified for seminal root number at low phosphorus availability (Table 1). A coincident QTL flanked by umc34/bn112.09 was detected for seminal root length at low phosphorus, which had an additive effect of 5.42 cm and an LOD value of 5.55. This coincident QTL explained 11.0% of phenotypic variation for seminal root length in the population at low phosphorus availability. Two QTL flanked by amp1/umc128 and bn112.09/umc131 were detected for seminal root length on chromosomes 1 and 2 under high phosphorus culture, which in combination explained 22.8% of the phenotypic variation in the population. Three QTL flanked by bn17.08b/amp1, bn112.09/umc131, and bn16.16/umc17 were also found for seminal root number at high phosphorus (Table 1).

QTL detected for these four traits are plotted in Fig. 3. Another interesting coincident QTL flanked by bn112.09/umc131 on chromosome 2 was also detected for seminal root length and number at high phosphorus. This coincident QTL explained 11.9% of phenotypic variation for seminal root length in the population at high phosphorus availability, and explained 7.0% phenotypic variation for seminal root number in the population (Table 1 and Fig. 3).

The allele at the locus flanked by umc34/bn112.09 on chromosome 2 had an additive effect of 5.42 cm for seminal root length at low phosphorus availability (Table 1). For seminal root length at high phosphorus availability, an allele on chromosome 1 had an additive effect of −3.34 cm, and an allele on chromosome 2 had an additive effect of 4.51 cm.

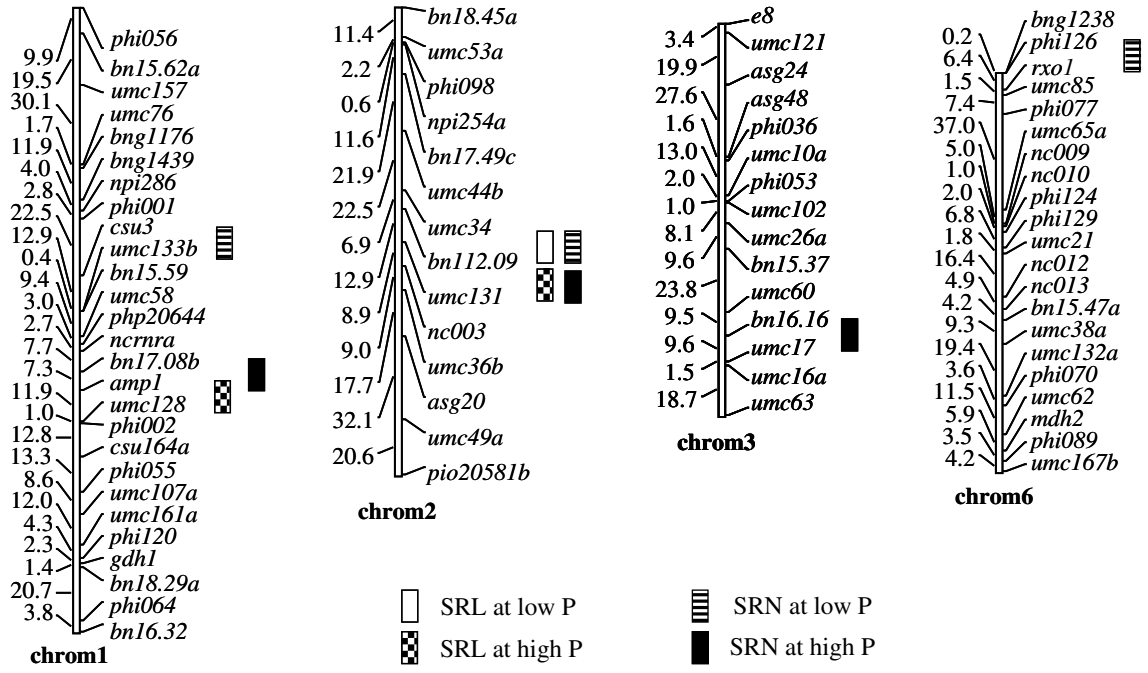

Fig. 3 QTL on chromosomes 1, 2, 3, and 6 for seminal root length (*SRL*) and seminal root number (*SRN*) in the RI population of B73×Mo17 at low- and high phosphorus (*P*) availability. The designation on the right is marker name, on the left is map distance based on the Kosambi function

Epistatic effects

Significant epistatic loci ($P<0.005$) for the four target traits were detected using QTL Mapper, a mapping program developed by Wang et al. (1999). Two pairs of epistatic loci for seminal root length at low phosphorus were identified on chromosomes 1, 2, 4, and 5, which together accounted for 23.2% of the total variation of this population (Table 2 and Fig. 4). Eight pairs of epistatic loci for seminal root number at low phosphorus were identified on chromosomes 1, 2, 3, 4, 5, 6, 7, 9, and 10, which together explained 50.6% of the total variation. Four pairs of epistatic loci for seminal root length at high phosphorus were identified on chromosomes 2, 5, 6, 7, 9, and 10, which together accounted for 32.2% of the total variation. Two pairs of epistatic loci for seminal root number at high phosphorus were identified on chromosomes 1, 2, 8, and 10, which together explained 20.3% of the total variation (Table 2 and Fig. 4). This demonstrated that the interaction of different loci can explain a portion of the genetic effect of the traits. One pair of epistatic QTL flanked by csu3/umc133b on chromosome 1, umc34/bn112.09 on chromosome 2, and phi126/rxo1 on chromosome 6, were also detected in the single QTL analysis (Tables 1, 2). A locus flanked by csu3/umc133b on chromosome 1 not only interacted with a locus flanked by nc007/umc147a on chromosome 5 for seminal root number at low phosphorus, but also with a locus flanked by umc34/bn112.09 on chromosome 2 for seminal root length at low phosphorus (Table 2 and Fig. 4). A locus flanked by php20725a/umc31a on chromosome 4 not only interacted with a locus flanked by umc147a/bng1219 on chromosome 5 for seminal root length at low phosphorus, but also with a locus flanked by umc147a/bng1219 on chromosome 5 for seminal root number at low phosphorus. Moreover, a locus flanked by umc34/bn112.09 on chromosome 2 interacted with three loci flanked by csu3/umc133b on chromosome 1 for seminal root length at low phosphorus, by npi333/php20608 on chromosome 4 for seminal root number at low phosphorus, and by bn15.62/umc157 for seminal root number at high phosphorus, respectively (Table 2 and Fig. 4).

Correlation and multiple regression analysis

Plant shoot dry weight in the field study was positively correlated with both seedling seminal root length and seminal root number in a controlled environment for 157 maize RILs from B73×Mo17 under low phosphorus availability (Fig. 5), although the coefficient of determinations ranged from 4 to 6.3% (Fig. 5). Multiple linear regression analysis showed that shoot dry weight in the field at low phosphorus (Y) $=5.28-1.13x_1$ (seed phosphorus content, mg) $-0.008x_2$ (root hair length at low phosphorus, mm) $+0.0118x_3$ (seminal root length at low phosphorus, cm) $+0.0218x_4$ (seminal root number at low phosphorus), which explained 24.6% of the variation in shoot dry weight in the populations.

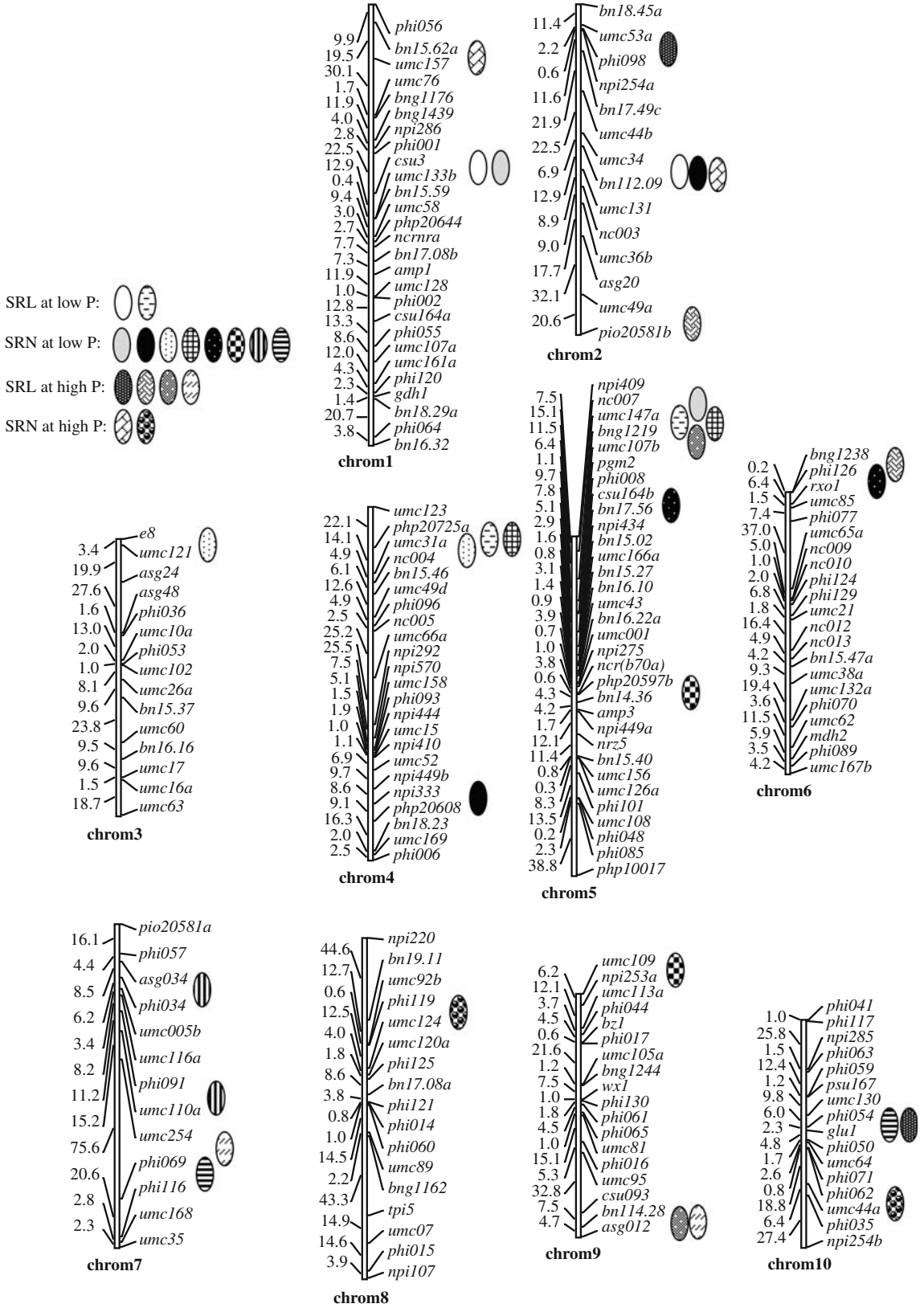

Fig. 4 Epistasis loci on chromosomes for seminal root length (*SRL*) and seminal root number (*SRN*) in the population at low- and high phosphorus availability. The designation on the right is marker name, on the left is map distance based on the Kosambi function

Table 2 Epistatic loci ($P < 0.005$) for seminal root length (SRL) and seminal root number (SRN) at low- and high phosphorus availability

Traits	Chromosome	Locus	Chromosome	Locus	LOD	Effect	R^2
At low phosphorus availability							
SRL	1	csu3/umc133b	2	umc34/bn112.09	8.37	−4.10	8.4
	4	php20725a/umc31a	5	umc147a/bng1219	5.83	5.46	14.8
SRN	1	csu3/umc133b	5	nc007/umc147a	5.00	−0.46	4.2
	2	umc34/bn112.09	4	npi333/php20608	7.14	0.49	4.8
	3	e8/umc121	4	umc31a/nc004	3.04	0.44	3.8
	4	php20725/umc31a	5	umc147a/bng1219	5.44	0.69	9.3
	5	csu164b/bn17.56	6	phi126/rxo1	6.54	0.24	10.8
	5	php20597b/bn14.36	9	umc109/npi253a	4.16	0.46	4.1
	7	asg034/phi034	7	phi091/umc110a	4.78	0.66	8.6
	7	phi069/phi116	10	phi054/glu1	3.19	−0.50	5.0
At high phosphorus availability							
SRL	2	umc53a/phi098	10	phi054/glu1	3.53	3.41	6.5
	2	umc49a/pio20581b	6	bng1238/phi126	3.00	3.13	5.4
	5	bng1219/umc107b	9	bn114.28/asg012	6.67	5.00	13.9
	7	umc254/phi069	9	bn114.28/asg012	3.37	−3.40	6.4
SRN	1	bn15.62a/umc157	2	umc34/bn112.09	3.50	−0.32	9.6
	8	phi119/umc124	10	phi062/umc44a	5.33	0.34	10.7

LOD $\log_{10}$-likelihood

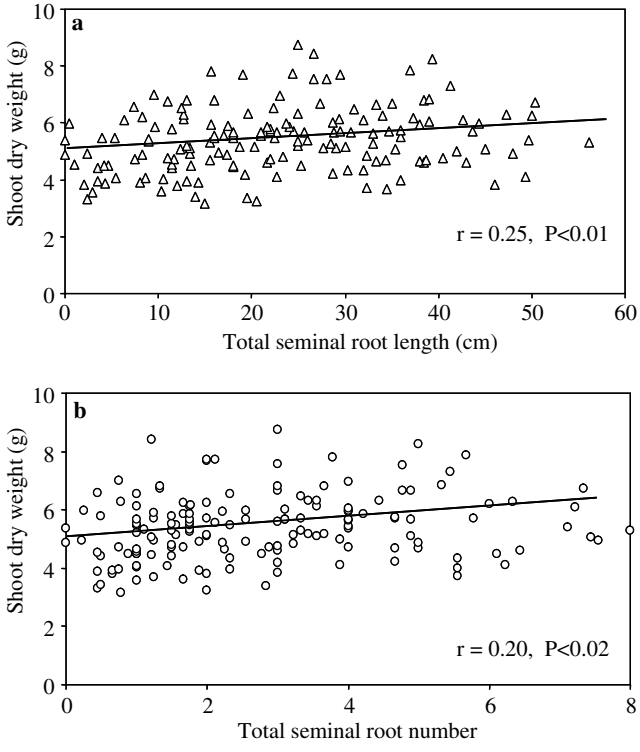

Fig. 5 Correlation of total seedling seminal root length and shoot dry weight (**a**), and total seedling seminal root number and shoot dry weight (**b**) for 157 maize RILs from B73×Mo17 under low phosphorus availability. Shoot weights were taken approximately 6 weeks after planting in five low phosphorus field environments. Five shoots per plot were harvested and dried for 1 week in forced air dryers before being weighed

Discussion

We observed substantial quantitative variation for seminal root length and number in the B73×Mo17 RIL population. Seminal roots are already present in the ungerminated mature maize kernel, which typically contains from 3 to 7 seminal root primordia (Feldman 1994). We identified 0 to 8 seminal roots at low phosphorus availability in the RI population, while under high phosphorus culture it ranged between 0.33 and 5.67. Seminal root length was increased by 38% at low phosphorus in the RI population. Moreover, seminal root length was increased by 32% in the phosphorus efficient genotype, Mo17 (Kaeppler et al. 2000). These results suggest an adaptive benefit of increased seminal root length for the uptake of immobile nutrients such as phosphorus by increasing soil exploration. Additionally, seminal root number was increased by 6.6% at low phosphorus in the RI population. This demonstrates that seminal root number is less stimulated by low phosphorus availability compared to seminal root length.

In this study, one main-effect QTL was associated with seminal root length, three QTL with seminal root number under low phosphorus, and two QTL with seminal root length, three QTL with seminal root number under high fertility, which accounted for 11.0, 25.4, 22.8, and 24.1% of phenotypic variation, respectively. A coincident QTL flanked by umc34/bn112.09 on chromosome 2 was found for both seminal root length and number at low phosphorus availability. The second coincident QTL flanked by bn112.09/umc131 on chromosome 2 was detected for both seminal root length and number at high phosphorus availability.

A QTL flanked by umc34 for seminal root length and number at low phosphorus availability is coincident with a QTL for mycorrhizal responsiveness of the shoot in maize (Kaeppler et al. 2000). A QTL flanked by umc128 on chromosome 1 for seminal root length and number at high phosphorus availability is coincident with a QTL for drought tolerance index and grain

yield under well-watered conditions (Tuberosa et al. 2002). A QTL flanked by umc131 on chromosome 2 for seminal root length and number at high phosphorus availability is coincident with a QTL for drought tolerance index and grain yield under drought (Tuberosa et al. 2002). A QTL flanked by bn16.16/umc17 for seminal root number at high phosphorus availability is coincident with a QTL for lateral root length of primary roots at low phosphorus in maize (Zhu et al. 2005b). No other QTL detected for seminal root traits in this study were coincident with QTL found in our previous studies on lateral rooting and root hair elongation at contrasting phosphorus supply (Zhu et al. 2005b, c). This is consistent with previous reports that distinct classes of roots may have distinct genetic control and response to environmental variables (Rundel and Nobel 1991; Lynch and van Beem 1993; Zobel 1996; Zhu et al. 2005b). Few QTL have been identified for root morphology traits in maize. Results reported here are consistent with QTL analysis of root traits in maize seedlings under cold stress. Hund et al. (2004) reported four distinct QTLs associated with lateral root length of the primary root and three distinct QTLs associated with seminal root length, indicating distinct QTLs controlling distinct root traits under cold stress. Moreover, no coincident QTLs were detected between these studies (Hund et al. 2004, Zhu et al. 2005b, c) and the study reported here.

Epistasis, or interlocus interaction, is a type of gene interaction whereby one gene interacts with the phenotypic expression of another non-allelic gene. The epistatic effects for maize seminal root length and number at different phosphorus levels are described in this study. This observation is consistent with a role for epistasis for specific combinations of maize inbred lines (Jinks 1955; Bauman, 1959, Darrah and Hallauer 1972; Lamkey et al. 1995; Wolf and Hallauer 1997; McMullen et al. 2001). In this study, two pairs of epistatic loci for seminal root length, eight pairs of epistatic loci for seminal root number at low phosphorus, four pairs of epistatic loci for seminal root length, and two pairs of epistatic loci for seminal root number at high phosphorus were identified, which accounted for 23.2, 50.6, 32.2, and 20.3% of the total variations, respectively; but no identical pairs were detected to be responsible for either seminal root length or seminal root number at low or high phosphorus availability. These results suggest that differential epistatic systems control seminal root elongation and development under different phosphorus availability, and that epistasis explains a large portion of the total genotypic variances of seminal root length and number. In the epistatic analysis, identical pairs were found to be responsible for seminal root number at low phosphorus and seminal root length at low phosphorus, with an additional pair of loci controlling seminal root length at low phosphorus. Moreover, a locus flanked by umc34/bn112.09 on chromosome 2 interacted with three loci flanked by csu3/umc133b on chromosome 1 for seminal root length at low phosphorus, by npi333/php20608 on chromosome 4 for seminal root number at low phosphorus, and by bn15.62/umc157 for seminal root number at high phosphorus, respectively. These results suggest that some epistatic loci control various types of phenotypic expression. These results also suggest the possibility of higher-order interaction (multi-locus epistasis) flanked by umc34/bn112.09 on chromosome 2. Epistatic effects are important when considering marker-assisted selection for seminal root trait-based breeding for more phosphorus efficient maize genotypes.

Neither seed size or seed phosphorus reserve was significantly correlated with seminal root length or number at either phosphorus level (data not shown). Similarly, there was no significant correlation between seed size or seed phosphorus reserve and root hair length (Zhu et al. 2005c). This demonstrates that the seminal root length and number may improve phosphorus efficiency regardless of seed size and seed phosphorus reserve.

The phenotypic data reported here were obtained from a controlled environment, and need to be confirmed in the field, although significant correlation was found between shoot dry weight and seminal root traits in the field at low phosphorus (Fig. 5). In the previous studies of root architecture and morphology in bean and maize, we have observed that genetic rankings, if not the absolute values for root parameters, are robust across environments (Miller et al. 2003; Liao et al. 2001; Zhu et al. 2005c; Rubio et al. 2003). However, root architectural traits may entail tradeoffs for other production constraints such as water acquisition (Ho et al. 2004, 2005). This set of RILs includes variation for several root traits, some of which may be synergistic for nutrient acquisition (Ma et al. 2001). Analysis of the importance of these QTL for phosphorus acquisition in the field might therefore be complex. This is consistent with our observation of no coincident QTL detected between the shoot dry weight and seminal root traits (data not shown).

Our analysis indicates that specific QTL explain significant variation for seminal roots among plants grown under both high and low phosphorus. Marker-assisted selection for seminal root traits may be useful to genetically improve maize root systems for uptake of more phosphorus without the tedious effort of phenotypic evaluation. Further comparisons need to be made to evaluate the consistency of QTL detection and epistasis for the same traits in various genetic backgrounds. This study indicates that substantial genetic variation exists for seminal root traits in maize, and the phenotypes are affected by nutrient level. The substantial number of epistatic loci controlling this trait indicates that complex pathways are likely involved in determining root length and number, especially under phosphorus deficiency. The results reported in this study will contribute to our ability to produce nutrient-efficient maize genotypes.

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