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Quantitative Trait Loci for Root Architecture Traits Correlated with Phosphorus Acquisition in Common Bean

Stephen E. Beebe, Marcela Rojas-Pierce, Xiaolong Yan, Matthew W. Blair,* Fabio Pedraza, Fernando Muñoz, Joe Tohme, Jonathan P. Lynch

ABSTRACT

Low soil P availability is a primary constraint to common bean (*Phaseolus vulgaris* L.) production in Latin America and Africa. Substantial genotypic variation in bean adaptation to low phosphorus (LP) availability has been linked with root traits that enhance the efficiency of soil foraging. The objectives of this study were to identify quantitative trait loci (QTLs) for P accumulation and associated root architectural traits, to facilitate genetic improvement and to reveal physiological relationships. Eighty-six $F_{5:7}$ recombinant inbred lines (RILs) were developed from a cross between G19833, an Andean landrace with high total P accumulation, and DOR 364, a Mesoamerican cultivar with low total P accumulation in LP conditions. A genetic map constructed with restriction fragment length polymorphisms (RFLPs), microsatellites, and PCR-based markers covering 1703 centimorgans (cM) total genetic distance and all eleven linkage groups (LGs) was used for QTL analysis. Seventy-one RILs were evaluated in the field at high phosphorus (HP) and LP for P accumulation, total root length (RL), specific RL, and plant dry weight (DW), while all 86 RILs were evaluated in a hydroponic system in the greenhouse for tap, basal, total, and specific RL and plant DW. Phosphorus accumulation in the field correlated with root parameters measured in the greenhouse. A total of 26 individual QTLs were identified for P accumulation and associated root characters using composite interval mapping (CIM) analysis. Phosphorus accumulation QTLs often coincided with those for basal root development, thus, basal roots appear to be important in P acquisition. Independent QTLs were identified for basal and taproot development, and for specific RL. Distinct QTLs for greater specific RL had positive, null and negative effects on P accumulation. Our results confirm the importance of root structure for LP adaptation and highlight the need for a more detailed understanding of root architectural traits for phenotypic as well as marker aided selection of more P-efficient crops.

PHOSPHORUS DEFICIENCY is a widespread nutrient constraint to crop production on tropical and subtropical soils that impacts millions of farmers, especially small landholders, on an area estimated at more than two billion hectares (Lynch, 1995, Fairhust et al., 1999). Correcting soil P deficiency with large applications of P fertilizer is not a viable option for most farmers in developing countries, and the inexpensive rock phosphate reserves remaining in the world could be depleted in as little as 60 to 80 yr. Therefore, sustainable P management in agriculture requires that researchers, plant breeders, and agronomists develop crops with enhanced P efficiency and management schemes that increase soil P availability (Vance, 2001).

The ability of a plant to access available P under LP conditions depends on its RL and on several other morphological and physiological properties of the root (Raghothama, 1999), including association with arbuscular-mycorrhizal fungi that increase the soil volume from which P can be acquired (Marschner, 1995, p. 889) and root-induced changes in the rhizosphere such as P mobilization by root exudates (Gaume et al., 2001). Superior P acquisition, often referred to as *phosphorus-acquisition efficiency*, differs from phosphorus-use efficiency, which is the plant's ability to produce yield per unit of acquired P from soil (Lynch and Beebe, 1995; Rao et al., 1999). Understanding the mechanisms and genetic control of phosphorus acquisition and use efficiency and other aspects of LP tolerance would facilitate genetic improvement (Lynch and Beebe, 1995; Rao, 2001). Root architectural traits that enhance topsoil foraging appear to be particularly important for P acquisition efficiency and genotypic adaptation of common bean to LP soils (Lynch and Brown, 1999, 2001). Phosphorus availability regulates many features of root architecture, including adventitious rooting, aerenchyma formation, basal root elongation, basal root-growth angle, lateral rooting, root hair density, and root hair length (Bates and Lynch, 1996; Bonser et al., 1996; Borch et al., 1999; Fan et al., 2003; Liao et al., 2001; Ma et al., 2001a; Miller et al., 2003). These changes appear to act synergistically to enhance P acquisition, by enhancing the quality and quantity of soil foraging, and by reducing the metabolic costs of soil exploration (Lynch and Ho, 2004; Lynch and Brown, 2001; Ma et al., 2001b). Another trait that varies with P supply is specific RL, defined as length of root per unit root weight (Miller et al., 2003). Specific RL is related to root diameter (Fitter, 1985; Eissenstat 1992) and root tissue density (Fan et al., 2003; Ryser, 1996), and is important in determining the metabolic cost of root elongation, an important aspect of efficient soil exploration (Lynch and Ho, 2004). Specific RL varies among species and cultivars (de Willigen and van Noordwijk,

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Abbreviations: AFLP, amplified fragment length polymorphism; CIM, composite interval mapping; cM, centimorgan; DW, dry weight; HP, high phosphorus; LG, linkage group; LOD, base 10 algorithm of the likelihood ratio; LP, low phosphorus; QTL, quantitative trait locus; R^2, proportion of variance explained by QTL at test site; RL, root length; SCAR, sequence characterized amplified region; RAPD, randomly amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; RIL, recombinant inbred line; TR^2, proportion of variance explained for the QTL and the background markers; TSP, triple super phosphate.

1987), and such differences have been associated with genetic differences in P efficiency (Sattelmacher et al., 1994).

Common bean is the most important food legume for direct human consumption in the world, and most production occurs in Latin America and Africa by resource-poor farmers on infertile tropical soils that are deficient in nutrients, especially P (CIAT, 1992). Genetic studies of common bean have concentrated on resistance to biotic constraints (Kelly et al., 2003), but several abiotic stress tolerances such as drought and LP tolerance have begun to be analyzed through a QTL approach (Schneider et al., 1997; Liao et al., 2004; Yan et al., 2004). Cultivated common bean was domesticated in the tropics and subtropics in at least two independent events (Gepts, 1988) and consists of two major gene pools, one Mesoamerican and one Andean, that display ample DNA polymorphism (Nodari et al., 1992). Differences have been observed in the ability of bean lines and landraces to produce grain under P limiting conditions (Thung, 1991; Youngdahl, 1990; Yan et al., 1995a; Beebe et al., 1997) and in their P acquisition efficiency (Yan et al., 1995b).

The objectives of this study were to identify QTLs for root architectural traits and evaluate their relationship with QTLs for P acquisition efficiency in common bean by analyzing a segregating population of RILs created from an intergene pool cross of common bean involving DOR 364, a genotype that is P inefficient, and G19833, a P-efficient genotype. This population has been used to analyze other LP adaptation traits such as root hair density, acid exudation, and basal root gravitropism (Liao et al., 2004; Yan et al., 2004).

MATERIALS AND METHODS

Parental Materials

Two genotypes, DOR 364 and G19833, were identified during several seasons of yield trials in the field and in controlled environments to be contrasting in growth, vigor, and yield under P-deficient conditions (Liao et al., 2001; Nielsen et al., 2001). DOR 364 is a small-seeded (21 g 100 seed^{-1}), high-yielding bred cultivar of indeterminate upright bush growth habit 2 (Schoonhoven and Pastor-Corrales, 1987) pertaining to race Mesoamerica of the Middle American gene pool as defined by Singh et al. (1991). It was developed in Central America for resistance to *Bean golden yellow mosaic virus* and is a widely grown commercial variety. DOR 364 was developed under fertile conditions and yields relatively poorly when P is limiting (CIAT, 1996, p. 22–38). G19833 is a Peruvian landrace called Chaucha Chuga, pertaining to race Nueva Granada of the Andean gene pool (Singh et al., 1991) that is large seeded (46 g 100 seed^{-1}), has type II growth habit, and yields nearly twice that of check cultivars under severe P stress where high temperature does not limit its adaptation (CIAT, 1991, p. 161–169). The cross of (DOR 364 × G19833) was created and 86 progenies were advanced by single seed descent to the F_5 generation. $F_{5,7}$ RILs were then increased for field studies. Seed weights of the RILs and parents were determined during the F7 generation before use in field and greenhouse trials described below.

Field Trials

Two trials were established in the field in Darién, Colombia (1400 m above sea level; 20°C average yearly temperature, Andosol soil type). The native soil P at this site is normally less than 2 mg kg^{-1} (Bray II). One trial was managed with LP levels (6 kg P ha^{-1} as triple super phosphate [TSP] before planting) and the other with HP levels (20 kg P ha^{-1} as TSP in both the season in which the trial was established and 6 mo previously). In both treatments, fertilizer was broadcast and incorporated. Parental genotypes were planted with six repetitions in each trial and 71 RILs for which seed was available were planted with three repetitions in a randomized complete block design. Ten seed were sown per experimental plot, which consisted of single rows 1 m in length and 0.6 m apart. At 35 d after planting, the plants in the extremes of the row were discarded and whole plants in the remaining 0.8 m of row were extracted manually from the soil with root systems conserved in the laboratory in sodium azide (0.02%) at 4°C and aerial parts separated from roots and both oven dried to determine shoot, root, and total DW.

Root Traits of Field-Grown Plants

Two randomly selected segments of 5 to 6 cm in length with attached lateral roots were cut from each conserved root system, and nodules were removed to avoid biasing estimates of root diameter. Samples were stained with neutral red (0.16 g L^{-1}), scanned and analyzed with Delta T-Scan software (Delta-T Devices, Burwell, Cambridge, UK). Data obtained were average root diameter and RL, as well as RL per diameter classes. Fragments were oven-dried and weighed to calculate specific RL as RL in meters per gram of root. Specific RL for the root sample were extrapolated to the whole root system on a weight–weight basis based on the harvest of the whole root system from the field. This gave us total RL and total length of fine roots (<0.38 mm). Plant tissue was analyzed for P concentration (Murphy and Riley, 1963) to calculate total P accumulation and P content per unit RL.

Root Traits of Greenhouse Grown Plants

Seed of the two parents and 86 $F_{5,7}$ RILs (including those in the field trial plus an additional 15) were surface sterilized for 1 min in 10% NaOCl before germination. Seed were germinated on germination paper soaked in 0.5 m*M* $CaSO_4$ in an incubator in the dark at 25°C. Seven days later, uniform seedlings were transplanted to the greenhouse at Pennsylvania State University with an average temperature of 29/20°C (day/night), a relative humidity of 48/83% (day/night), and an average measure of photosynthetically active radiation between 500 and 1000 μmol m^{-2} s^{-1}. Plants were grown at a low level of available P (0.2 μ*M* P) in 100-L hydroponic tanks with nutrient solution containing (in m*M*) 4.5 KNO_3, 1.2 NH_4NO_3, 3.6 $Ca(NO_3)_2$, 3.0 $MgSO_4$, 1.2 K_2SO_4, 1.2 $(NH_4)_2SO_4$, and (in μ*M*) 30 Fe-EDTA, 4.5 $MnSO_4$, 4.5 $ZnSO_4$, 1.5 $CuSO_4$, 1.5 H_3BO_3, and 0.4 $NH_4Mo_7O_{24}$. The solution was well aerated and the pH was maintained between 5.8 and 6.0 with daily additions of 1.0 *M* KOH or HCl. Plants were harvested 14 d after transplanting and divided into leaves, stems, and roots. The roots were conserved in 25% ethanol immediately after harvest and then divided into tap and basal roots, and basal roots were counted. The roots were stained with 0.5 m*M* neutral red dye (Sigma, USA) before being scanned into images with a desk scanner (ScanJet IIc, Hewlett-Packard, USA). Samples were dried and weighed to determine basal root DW, taproot DW, total root DW, specific RL, shoot DW, and total DW. Knowing average root diameter and length surface area

was estimated by geometry. From the images, tap RL, basal RL, and total RL were analyzed by computer as described above with the DT-Scan program (Delta T, Inc., Richfield, WI).

Map Construction

DNA was extracted from parental genotypes by a modified Dellaporta method used by Vallejos et al. (1992). Parental polymorphism surveys were prepared by digesting the parental DNA with six restriction enzymes (*Bam*HI, *Dra*I, *Hind*III, *Eco*RI, *Eco*RV, and *Xba*I) and transferring the digested DNA to Nylon membranes. Southern hybridization was conducted with 101 restriction fragment length polymorphism (RFLP) probes from two common bean genetic maps (Vallejos et al., 1992; Nodari et al., 1993). Fifty probes were chosen based on polymorphism among the parents and map position in the genome and were evaluated on the 86-RIL progeny. Additionally, a selection of 32 microsatellites from Yu et al. (2000) and Blair et al. (2003) were amplified on the DNA of the RILs. Two amplified fragment length polymorphism (AFLP) primer combinations, used previously to produce a large number of bands in common bean (Tohme et al., 1996), were employed to generate 24 additional markers. In addition, sequence characterized amplified region (SCAR) primer pairs developed by Gu et al. (1995) were used to amplify another six bands and a total of 23 10-mer oligonucleotide primers from Operon (Huntsville, AL) were used to generate 124 randomly amplified polymorphic DNA (RAPD) markers. Markers presenting significant segregation distortion were eliminated. A total of 236 markers were used to create the final map, extending a map that was reported previously (Beebe et al., 1998) using the Map Maker (Kintyre, UK) program (Lander et al., 1987). Linkage analysis was conducted initially for the RFLP and microsatellite markers at likelihood of odds (LOD) 6 to anchor the map to the core map of Freyre et al. (1998). Remaining markers were placed individually at a LOD > 3.5 using the Assign and Place functions and confirmed by the Ripple function in Map Maker.

Statistical Analysis

Analyses of variance were performed using the SAS program (SAS Institute, 1987) for both field and greenhouse traits. Since the parental genotypes were planted with more repetitions than the RILs in the field, they were analyzed separately from the RILs in all the trials. Simple linear correlations were calculated among mean values of variables in both the field and greenhouse trials to reveal physiological relationships. The QTLs were detected with CIM analysis, which was performed using the software program QTL Cartographer v. 1.15 (Basten et al., 2005). Since our interest was primarily P accumulation in P-limited conditions, our analyses focused on the association of root variables with P accumulation in the low-P field trial. The following traits were analyzed singly and jointly with P accumulation in the LP treatment: total RL and specific RL in the low-P field trial; basal RL and DW in the greenhouse trial; and tap RL and DW in the greenhouse trial. In the SRmapQTL subprogram, parameters for forward–backward stepwise regression analysis were a window size of 10 cM, a walkspeed at every 1 cM, and probability thresholds of 0.05 for the partial F test for both marker inclusion or exclusion. The five most significant markers found with the SRmapQTL subprogram were used as background markers in the single and joint CIM analysis realized with the ZmapQTL and JZmapQTL subprograms, respectively. In the single CIM analysis, determination coefficients were calculated for each interval separately (R^2) and for each interval given the background markers (TR^2). The subprogram Eqtl was used to summarize the significant QTLs found with the previous CIM subprograms. The LOD thresholds were set at a default of 2.5 for both the individual and joint analysis. The LOD thresholds were also calculated through the generation of 1000 permutations (Churchill and Doerge, 1994), to determine an effective significance level of $P = 0.05$ across the genome. Results were displayed using QTL Cartographer v. 1.21 and represented graphically with standard drawing software, to designate genomic regions that proved to be significant in the analysis described above.

RESULTS

Field and Greenhouse Results with Parental Genotypes

The G19833 parent acquired more P than the DOR-364 parent, although shoot DW did not necessarily reflect the larger root systems found in G19833 (Table 1). At HP, the two parental genotypes had similar values for shoot, root, and total DW, and although G19833 tended to have greater values for other root parameters such as total root surface, differences were not significant. At LP, the parental genotypes were differentiated principally on root parameters (e.g., total root weight, RL, root surface, length of fine roots). G19833 produced three times the RL of DOR 364 at LP, and these roots had greater specific RL ($P = 0.05$). G19833 produced nearly the same total RL at LP as at HP, while DOR364 had much less RL under P deficiency. G19833 acquired 59% more P than DOR 364 at LP, and 84% more P at HP (Table 1), although this effect was significant only at HP.

The effect of P fertilization on plant response was evident in the two field trials managed with different levels of P. Averaged across parents, shoot, root, and total DW accumulation in the HP treatment were 157.2, 127.5, and 145.5% higher than at LP (Table 1), while RL, root surface, and root diameter were 41.4, 78.7, and 113.9% higher in HP than in LP treatments. Total P accumulation and P accumulation per unit RL were 328 and 245% higher in the HP treatment than in the LP treatment, as would be expected from the greater nutrient supply in the HP treatment. In contrast, length of fine roots was unchanged between the treatments and specific RL was only 39.6% higher in the LP treatment.

In the greenhouse study, differences were observed between the two parents for root traits that were comparable with differences observed in the field at LP (Table 2). G19833 had significantly higher total RL, basal RL, and DW than DOR364 in the greenhouse. The two parents were not significantly different for specific RL nor for taproot parameters. Unlike the field trial, G19833 had more DW both in shoots and basal roots, as can occur with large-seeded beans at early stages of development (Yan et al., 1995b).

Field and Greenhouse Results with Progenies

In the field trials, the response of the progeny lines to LP was similar to that of the parents, although greater

Table 1. Significance of ANOVA mean squares for means of root and other parameters of two common bean genotypes and their 71 recombinant inbred line progenies in two field trials under different P levels.

	Low P				High P			
Trait	G19833	DOR 364	Differences among parents	Differences among lines	G19833	DOR 364	Differences among parents	Differences among lines
Total DW, g plant^{-1}†	5.50	7.52	ns‡	**	17.36	16.14	ns	***
Total shoot DW, g	2.82	5.37	ns	*	8.78	9.88	ns	**
Total root DW, g	3.22	2.14	*	***	6.91	6.25	ns	***
Total P accumulation, mg plant^{-1}	9.89	6.22	ns	ns	44.74	24.31	*	*
Total RL, m plant^{-1}§	40.82	12.58	*	***	49.25	26.24	ns	***
Total root surface, cm^2 plant^{-1}	46 144	16 023	*	*	78 391	32 716	ns	***
Average diameter, mm	0.36	0.41	ns	ns	0.39	0.43	ns	(0.06)
Length of fine roots, m	34.94	8.15	*	***	25.40	19.19	ns	***
Specific RL, m g^{-1} root	128.6	69.5	*	ns	76.0	45.6	ns	ns
P uptake per unit RL, mg m^{-1}	0.20	0.55	ns	***	1.36	1.23	ns	ns

* Statistically significant at the 0.05 level.
** Statistically significant at the 0.01 level.
*** Statistically significant at the 0.001 level.
† DW = dry weight.
‡ ns = not significant.
§ RL = root length.

Table 2. Significance of ANOVA mean squares for root and other parameters of two common bean genotypes and their 86 recombinant inbred line progenies under phosphorus stress in a greenhouse hydroponic system.

Trait	G19833	DOR 364	Differences among parents	Differences among lines
Total DW, g plant^{-1}†	1.15	0.58	*	***
Shoot DW, g plant^{-1}	0.91	0.43	*	***
Total root DW, g plant^{-1}	0.24	0.14	ns‡	***
Total RL, m plant^{-1}§	68.15	34.48	*	***
Basal root DW, g plant^{-1}	0.19	0.07	*	***
Basal RL, cm plant^{-1}	52.36	14.79	*	***
Basal root no. plant^{-1}	8.33	2.67	ns	***
Taproot DW, g plant^{-1}	0.05	0.07	ns	***
Tap RL, cm plant^{-1}	15.81	19.69	ns	**
Specific RL, m g^{-1}	281.7	243.3	ns	ns

* Statistically significant at the 0.05 level.
** Statistically significant at the 0.01 level.
*** Statistically significant at the 0.001 level.
† DW = dry weight.
‡ ns = not significant.
§ RL = root length.

degrees of freedom permitted the detection of effects at a higher level of confidence than for the parents (Table 1). Similarly, in the HP field trial, a greater number of traits showed significant effects in the progeny lines than in the parents. Among traits displaying significant differences, coefficients of variation ranged from 36 to 50%. Meanwhile, in the greenhouse trial, every parameter except for specific RL had significant differences among the progeny lines, including those related to taproot development, for which a significant difference was not detected in the parents (Table 2).

Phosphorus accumulation of field-grown progenies was correlated with some root traits in both HP and LP environments, including basal root DW and basal RL; however, total RL was only correlated with P accumulation in the HP environment, not in the LP environment (Table 3). Correlations of P accumulation in the field with root data in the greenhouse were significant, especially with basal RL and DW (r = 0.351–0.378, P = 0.001), but also with total RL and root DW (data not shown).

Analysis of Quantitative Trait Loci

The level of polymorphism between DOR 364 and G19833 was comparable with levels previously reported for populations from crosses between Mesoamerican and Andean beans (Nodari et al., 1992; Vallejos et al., 1992). The single copy RFLP and microsatellite markers were used to create a high LOD framework map that, combined with the AFLP and RAPD markers, represented the 11 LGs of the *Phaseolus* genome (Beebe et al., 1998; Blair et al., 2003) and had a total length of 1703 cM, giving an average distance between markers of 7.2 cM that was useful for QTL analysis. The LGs and order of RFLP and microsatellites were as reported previously (Vallejos et al., 1992; Blair et al., 2003) and could be readily correlated with the integrated map for the species (Freyre et al., 1998). Linkage group designation and orientation are as per that combined map.

A total of 29 significant QTLs for the individual traits were identified with CIM and were named according to a three-letter convention that was numbered with the LG and QTL order (Table 4). Two significant QTLs were identified for P accumulation in the LP treatment analyzed as an individual trait on LGs B4 and B10 (Fig. 1). The QTLs for RL and for specific RL were found in the same regions as these two loci, respectively. Different QTLs were identified for P accumulation, RL and specific RL in the HP treatment than in the LP treatment. For example, a very significant QTL for P accumulation in the HP environment was found on LG B02 at a site that did not contain QTLs for other traits in the LP environment. Similarly, the QTLs for specific RL in the high-P environment were on separate LGs compared with the QTLs for this trait in the low-P environment. On the other hand, the QTLs for RL on LGs B4 and B7 in the HP environment were in equivalent positions to the QTLs for this same trait on these LGs for the LP environment. A total of three additional RL, three seed weight, two basal RL, three basal root DW, three specific RL, one taproot RL, and three taproot DW QTLs were also identified on six additional LGs, B1, B3, B7, B8, B9, and B11, in the greenhouse

Table 3. Correlations among root and P uptake traits for recombinant inbred lines of DOR 364 × G19833 in field and greenhouse trials.†

	Field traits				Greenhouse traits		
	Total RL		P uptake		Basal root		Taproot
Trait	LP	HP	LP	HP	DW	RL	DW
Total RL, HP field	0.361***						
P uptake, LP field	0.225ns	0.204ns					
P uptake, HP field	0.317**	0.308**	0.349***				
Basal root DW	0.461***	0.442***	0.367***	0.378***			
Basal root RL	0.384***	0.287*	0.357***	0.315**	0.845***		
Taproot DW	−0.04ns	0.167ns	0 ns	0.147ns	0.003ns	−0.21ns	
Taproot RL	0.015ns	0.123ns	0.005ns	0.076ns	−0.03ns	−0.14ns	0.887***

* Statistically significant at the 0.05 level.
** Statistically significant at the 0.01 level.
*** Statistically significant at the 0.001 level.
† DW = dry weight; HP = high-phosphorus treatment and LP = low-phosphorus treatment, as applied to RL and P accumulation; RL = root length.
‡ ns = not significant.

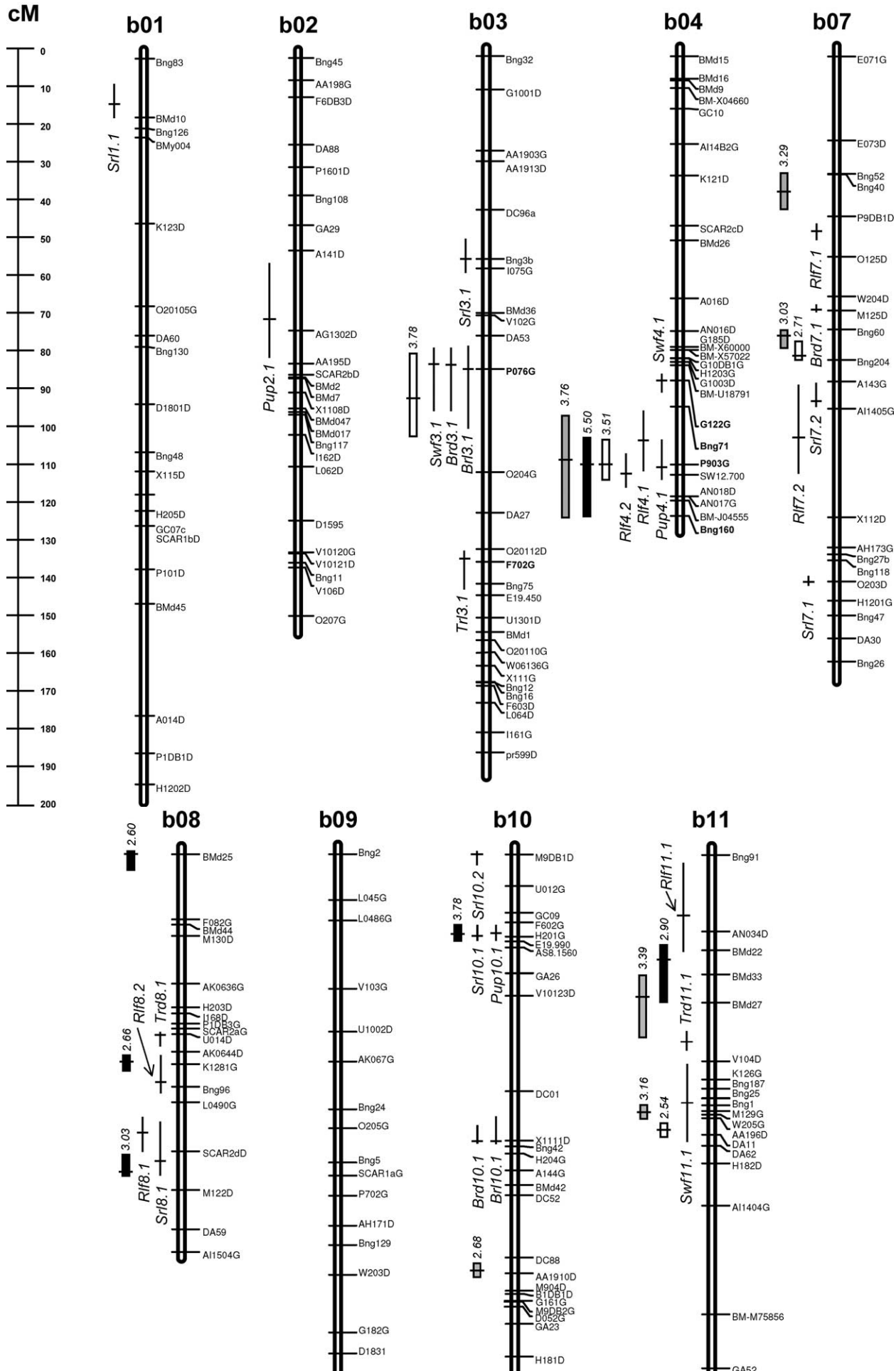
Table 4. Quantitative trait loci (QTLs) revealed by composite interval mapping analysis of individual traits from the field and greenhouse evaluations of the DOR364 × G19833 recombinant inbred line population. Values represent QTL significance (LOD) and determination coefficients explained by each QTL (R^2 and TR^2). Linkage group location and nearest marker to the peak LOD value are given for each QTL.†

Trait	Experiment‡	QTL name	LG	Nearest marker	LOD§	R^2	TR^2	Increased effect
P accumulation, mg plant^{-1}	field, LP	*Pup4.1*	B4	P903G	3.15	0.1341	0.4929	G19833
	field, LP	*Pup10.1*	B10	F602G	3.16	0.1405	0.5019	DOR364
Root length, m plant^{-1}	field, LP	*Rlf4.1*	B4	Bng71	4.17	0.2060	0.6121	G19833
	field, LP	*Rlf7.1*	B7	O125D	2.68	0.1033	0.6111	G19833
	field, LP	*Rlf8.1*	B8	SCAR2dD	3.81	0.2500	0.6464	DOR364
	field, LP	*Rlf8.2*	B8	Bng96	5.61	0.2141	0.6105	DOR364
Specific root length, m g^{-1} root	field, LP	*Srl8.1*	B8	SCAR2dD	4.34	0.2023	0.5832	DOR364
	field, LP	*Srl10.1*	B10	H201G	4.23	0.1913	0.4181	G19833
P accumulation, mg plant^{-1}	field, HP	*Pup2.1*	B2	AG1302D	6.58	0.5133	0.7158	G19833
RL, m plant^{-1}	field, HP	*Rlf4.2*	B4	SW12.700	3.37	0.1242	0.4918	G19833
	field, HP	*Rlf7.2*	B7	AI1405G	4.70	0.3742	0.6976	G19833
	field, HP	*Rlf11.1*	B11	AN0304D	4.78	0.3050	0.6949	G19833
Specific RL, m g^{-1} root	field, HP	*Srl3.1*	B3	Bng3b	3.81	0.1730	0.4295	G19833
	field, HP	*Srl7.2*	B7	AI1405D	3.52	0.1594	0.4298	G19833
Basal RL, cm plant^{-1}	greenhouse	*Brl3.1*	B3	P076G	5.87	0.1997	0.4691	DOR364
	greenhouse	*Brl10.1*	B10	X1111D	3.80	0.1197	0.4696	G19833
Basal root DW, g plant^{-1}	greenhouse	*Brd3.1*	B3	P076G	3.66	0.1138	0.4811	DOR364
	greenhouse	*Brd7.1*	B7	M125D	2.91	0.0885	0.4823	G19833
	greenhouse	*Brd10.1*	B10	X1111D	2.99	0.0899	0.4825	G19833
Taproot RL, cm plant^{-1}	greenhouse	*Trl3.1*	B3	F702G	4.86	0.2530	0.4829	G19833
Taproot DW, g plant^{-1}	greenhouse	*Trd8.1*	B8	U014D	4.34	0.1463	0.4424	G19833
	greenhouse	*Trd9.1*	B9	Bng65	4.75	0.2242	0.5118	G19833
	greenhouse	*Trd11.1*	B11	BMd27	2.86	0.1392	0.5095	G19833
Specific RL, m g^{-1} root	greenhouse	*Srl1.1*	B1	BMd10	4.15	0.2462	0.4801	DOR364
	greenhouse	*Srl7.1*	B7	AI1405G	2.59	0.0944	0.3649	G19833
	greenhouse	*Srl10.2*	B10	M9DB1D	3.24	0.1202	0.3649	G19833
Seed weight, g 100^{-1} seed	NA¶	*Swf3.1*	B3	P076G	3.94	0.1023	0.5768	DOR364
	NA	*Swf4.1*	B4	G122G	3.08	0.0878	0.6598	G19833
	NA	*Swf11.1*	B11	Bng1	7.50	0.2163	0.5814	G19833

† DW = dry weight; LG = linkage group as defined by Freyre et al. (1998); R^2 = proportion of variance explained by QTLs at test site; RL, root length; TR^2, proportion of variance explained for the QTLs and the background markers.
‡ HP, high phosphorus treatment; LP, low-phosphorus treatment.
§ LOD (base 10 algorithm of the likelihood ratio) threshold of 2.5 used for QTL detection. Empirical LOD thresholds based on 1000 permutations as recommended by Churchill and Doerge (1994) were 3.45 for P accumulation (field, LP), 3.60 for root length (field, LP), 3.37 for specific root length (field, LP), 3.55 for P accumulation (field, HP), 3.73 for root length (field, HP), 3.22 for specific root length (field, HP), 3.19 for basal root length (greenhouse), 3.38 for basal root DW (greenhouse), 3.41 for taproot length (greenhouse), 3.28 for taproot DW (greenhouse), 3.24 for specific root length (greenhouse), and 3.38 for seed weight.
¶ NA = not applicable.

environment. One of the QTLs for specific RL in the greenhouse was also linked to a QTL for the same trait in the HP environment on LG B7. Another QTL for specific RL in the greenhouse was in the same position on LG B10 as a QTL for specific RL in the LP environment. Quantitative trait loci for different traits were also located together at similar regions of the genome. For example, regions on LGs B3 and B10 contributed simultaneously to both basal root DW and length, while other associations are described further below. The highest LOD score for any QTL was 7.5, although QTLs for P accumulation tended to be of lower significance. The determination coefficients for the QTLs identified by individual CIM analysis ranged from 8.9 to 25.0% individually (R^2), and from 41.8 to 66.0% when evaluated with background markers (TR^2) (Table 4). All QTLs had markers within 5 cM of the highest LOD peak (Fig. 1).

Alleles from both parents were associated with increases in different sets of traits at different QTLs for the LP and greenhouse environments. In the case of taproot development, QTLs on B8 and B9, the alleles



from G19833, caused increases in tap RL and DW. For P accumulation in the LP treatment, the positive allele for the QTL on LG B4 was from G19833 as expected; however, the positive allele for the QTL on LG B10 was from DOR364. In the case of specific RL QTLs, the positive alleles for two QTLs on B08 were from DOR 364, while the positive allele for the QTL on LG B10 was from G19833. In the greenhouse, the increased effect of the specific RL QTLs were from both DOR364 (on LG B1) and G19833 (on LGs B7 and B10). Therefore, the QTL region on B10 had a double effect, whereby an allele or gene from G19833 increased specific RL, and an allele or gene from DOR 364 increased P accumulation in the LP treatment. The alleles for increased P accumulation, RL, and specific RL for the QTLs for these traits in the HP environment were all derived from G19833.

In the joint QTL analysis, CIM was used to identify regions of the genome where interactions between P accumulation under LP conditions and other traits were significant. Results of three of these analyses (with basal RL in the greenhouse; specific RL in the field; and total RL in the field) are presented. The joint analysis of traits identified not only the QTLs on B04 and B10 which directly influenced P accumulation in the LP treatment, but also several others where there was an association between P accumulation in the field and specific root traits. Quantitative trait loci regions for P accumulation were most consistently associated with basal root DW and length in the greenhouse, and with RL in field. For example, a QTL on LG B04, close to the RAPD marker P903G, was associated with increased P accumulation in the LP treatment, while the joint analysis of this trait plus basal RL in the greenhouse or total RL in the LP field resulted in more significant QTLs close to the same marker (peak LOD = 3.7) compared with the individual analysis (peak LOD = 3.1). Similar relationships were inferred from the joint analysis of P accumulation in the LP trial and basal or total RL traits, which showed regions within LGs B03, B07, and B11 that had significant QTLs (these latter two LGs with two separate regions each).

A positive relationship between specific RL and P accumulation in the LP trial was uncovered by the joint QTL analysis, in which we found significant regions on B04 and B11 for this combination of traits. The region on LG B04 was interesting because it was also significant for the combined analysis of basal roots plus P accumulation in the LP treatment, suggesting that basal roots and total RL contributed to finer roots and greater specific RL. At all of these loci, the increased effect of the individual traits was derived from G19833. For three more regions on B08, significance in the joint analysis was not greater than that of specific RL alone, and the positive effect on specific RL was derived from DOR 364. In still another region on B10, the relationship of specific RL and P accumulation was different. Here, the segment that contributed to specific RL was not associated with either basal root development or taproot development, but coincided with *Pup10.1*, the most important QTL for P accumulation under the LP trial

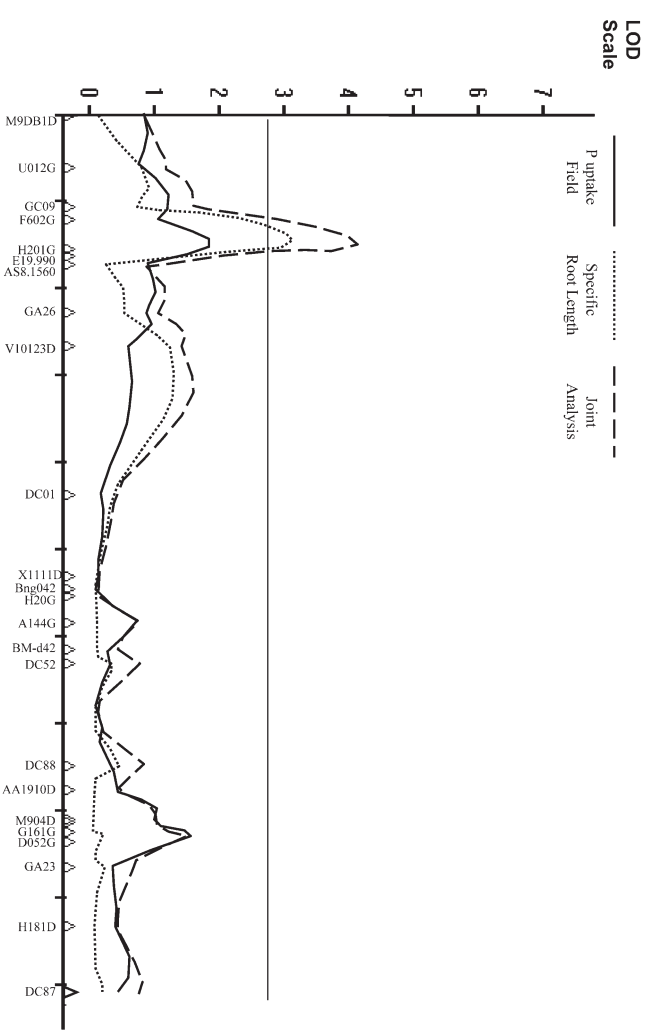


Fig. 2. Significance of joint and individual quantitative trait loci (QTLs) for specific root length and P accumulation on Linkage Group B10. Results are represented as a likelihood of odds trace from composite interval QTL mapping analysis. Genetic markers and distances are as in Fig. 1.

(Fig. 2). Greater significance of this QTL was observed in the joint analysis (peak LOD = 3.8) than for P accumulation alone (peak LOD = 3.15); thus, joint analysis strengthened the conclusion suggested by single trait analysis that thicker roots were indeed associated with greater P accumulation efficiency at this QTL.

In both the individual and joint analysis, QTLs for taproot DW and length in the greenhouse did not coincide with those for basal roots or P accumulation under LP treatment. Regions in B08 and B09 were found to affect taproot DW, and another on B03 was correlated with tap RL. Even in these cases, the joint analysis suggested no direct relationship of taproot with P accumulation, since the significance of QTLs for taproot DW or length plus P accumulation under LP treatment did not increase over that of the individual parameters (data not shown). Apart from root or P accumulation characteristics, a total of three QTLs were found for increased seed size under the LP field trial. Interestingly, all three of these QTLs were associated with QTLs for root characters and increased P accumulation. Such an

association was evident in LGs B03 and B11 for basal root parameters. While on B04, QTL for seed size (*Swf4.1*) and for P accumulation in the LP trial (*Pup4.1*) were linked. Of the three seed weight QTLs, the QTLs on LGs B03 and B04 may be associated with QTLs found on these LGs in previous studies of the inheritance of seed weight in common bean (Kelly et al., 2003).

DISCUSSION

Root traits are important for crop adaptation to edaphic stress. This is especially true for low soil P availability, since P acquisition is strongly dependent on soil exploration and root architecture (Barber, 1995). A significant challenge to selection for root traits is the difficulty of evaluating root phenotypes, since many root traits are phenotypically plastic, roots are difficult to extract from the soil, such extraction may change certain traits such as architecture, and many root sampling procedures are destructive. These challenges make the prospect of marker aided selection an attractive alternative to phenotypic selection. Genetic mapping is also helpful in understanding the complexity of genetic control of root traits of interest, and in revealing or validating functional relationships between specific root traits and other traits of interest such as yield in LP soils. The utility of genetic mapping in revealing such functional relationships is valuable for root traits, since relatively little is known about the importance of root traits for crop adaptation to edaphic stresses. DNA markers have been utilized to study root traits or to explore their relationship to LP tolerance in rice (*Oryza sativa* L.) (Champoux et al., 1995; Price et al., 1997; Price and Tomos, 1997; Wissuwa and Ae, 2001; Wissuwa et al., 1998, 2002) and maize (*Zea mays* L.) (Zhu et al., 2004). In common bean, we have used QTL analysis to show the importance of root hairs and rhizosphere acidification (Yan et al., 2004) as well as basal root shallowness (Liao et al., 2004) for LP adaptation in common bean. In this study, we used QTL analysis to further define root traits associated with P accumulation that would facilitate improvement of LP adaptation, obviating the need for direct evaluation of root systems.

The two parental genotypes used in this study contrasted for root traits and LP adaptation (P efficiency). Previous studies show that G19833 has superior growth and yield in LP soil field trials than DOR 364 (CIAT, 1991, p. 161–169; Liao et al., 2004), and superior growth under LP stress in soil, P-buffered sand, and growth pouches in controlled environments (Liao et al., 2001; Liao et al., 2004; Yan et al., 2004). The distinct evolutionary background of these two materials, with DOR364 derived from the Mesoamerican gene pool and G19833 a landrace of the Andean gene pool, may have contributed to the physiological differences that were observed between them. G19833 has greater expression of several root traits that contribute to efficient P acquisition in LP environments, including greater root hair length and density (Yan et al., 2004), greater root exudation of acid (Yan et al., 2004), more shallow deployment of basal roots (Liao et al., 2004), greater cortical aerenchyma formation (Fan et al., 2003), and greater production of adventitious roots (Miller et al., 2003). These traits confer on G19833 the ability to exploit topsoil P resources more effectively than DOR 364, which is an important aspect of LP adaptation in bean (Lynch and Brown, 2001), and reduce the metabolic cost of soil exploration in G19833, which is another important component of LP adaptation in bean (Lynch and Ho, 2004). Data presented here are consistent with these earlier observations, showing that among RILs descended from G19833 and DOR364, P acquisition in the field is linked with root growth, and specifically with basal root development and specific root length. In this study, while RL in the field had low correlations with P accumulation, perhaps because of variability in plant development under field conditions, or error introduced in the extraction of roots from soil, the traits basal root DW and length in the greenhouse hydroponic trial presented higher correlations with total RL and P accumulation in the low and HP field environments. The correlation of root traits expressed early in plant development, such as basal roots with eventual yield under LP conditions, is consistent with earlier reports that basal root angle of young seedlings is well correlated with field performance under LP conditions (Bonser et al., 1996; Rachier et al., 1998). Basal roots emerge from the primary root within 1 wk of germination, and establish the structural scaffold for the development of the majority of the mature root system under normal circumstances. Basal root development in seedlings may therefore be useful indicators of root development in the field, as shown here. It is noteworthy that as in the report by Bonser et al. (1996), basal root growth as assessed in a controlled environment has meaningful correlation with field performance despite the well-known phenotypic plasticity of root traits.

Quantitative trait locus analysis revealed a relationship of P accumulation with greater RL in the field and with basal root DW or RL in the greenhouse. This latter relationship was consistent across six different chromosomal regions on five LGs. Therefore, QTL analysis was in agreement with phenotypic correlations that associated P accumulation with basal roots and supported the conclusion that basal roots play a very important role in P accumulation by bean. This could be explained by the hypothesis that basal roots tend to explore more superficial soil layers, where soil P availability is greater than in subsoil layers (Lynch and Brown, 2001). Quantitative trait locus analysis also identified genetic linkage between the three QTLs for seed weight and several root trait QTLs. This may explain a relationship between seed size and LP tolerance that was noted previously, and was attributed to greater seed P and carbon reserves of large-seeded beans, which resulted in better growth early in plant development (Yan et al., 1995b). The question of an association between seed size QTLs and root trait QTLs deserves more attention, especially regarding Andean beans that tend to have larger seed.

Specific RL, as a measure of root fineness, is generally expected to be positively correlated with efficient P

acquisition, since theoretically finer roots should be more efficient in exploring the soil per unit of metabolic investment in root biomass (Eissenstat, 1992). Although finer roots may incur tradeoffs in terms of increased root mortality and susceptibility to pathogens and herbivores, a recent study of root longevity and turnover in bean under LP stress found low rates of root mortality (Fisher et al., 2002), suggesting that such tradeoffs are not of central importance in common beans. In addition to root diameter, specific RL could be influenced by anatomical traits that change the relationship of root volume and root weight. Cortical aerenchyma formation is particularly interesting in this regard, since LP stress stimulates aerenchyma formation in bean, thereby reducing the metabolic costs of soil exploration, and since G19833 has more cortical aerenchyma under P stress than DOR364 (Fan et al., 2003). Another factor influencing bulk root system specific RL is root architecture and branching. The root system is composed of several distinct types of roots, including the taproot, basal roots, adventitious roots, and their laterals, which have substantially different diameters, tissue densities, and therefore specific RL (Lynch and van Beem, 1993; Miller et al., 2003) which could also have contributed to bulk differences in specific RL.

In the QTL analysis we found contrasting effects of specific RL between genotypes from the two gene pools of common beans. For at least two QTLs, greater specific RL contributed to P accumulation as expected, but at other QTLs its effect was null, or even negative, as in the case of the QTL *Srl10.1* on B10. The relationship of specific RL and P accumulation at this locus was suggestive of a pleiotropic QTL or cluster of linked QTLs that would control both traits and that the alleles for thicker roots were associated with the alleles for increased P acquisition. This implies a different mechanism for P accumulation in DOR364 than in G19833, whereby DOR364 tends to express greater P accumulation per unit RL than G19833. This effect was not significant between the parents, but the progenies did show different amounts of P accumulation per unit RL, suggesting that this trait had a genetic and physiological basis. The efficiency of fine roots vs. course roots per unit of P accumulation and the mechanism of P accumulation in DOR364 require additional study to determine the most appropriate root structure at a given P level. The effect of longer rooting systems appears to be important for P accumulation as suggested by the association of the *Pup4.1* QTL with RL QTLs identified on LG B4 for both high and LP environments. The positive allele for all of these QTLs was derived from G19833 the more LP tolerant parent suggesting that this region of the genome can be used for improvement of LP tolerance. The relationship of RL QTLs to specific RL QTLs that were found in the same regions of the genome also would be of interest to analyze in greater detail to determine if longer root systems are associated with finer rooting.

Apart from the relationship of these root traits to P accumulation, a more general observation concerns the complexity of root systems and their genetic control. The present study offers a comparison of QTL mapping positions for several root traits in bean that have not been reported previously, and identifies almost thirty individual QTLs in 15 different regions of the genome spread across nine LGs which affect root traits. Some QTLs were associated with both basal and taproots, and others only with one or the other, while some QTLs for these traits were associated with specific RL and others were not. Quantitative trait loci for P accumulation in the LP environment on LGs B4 and B10 were associated with QTLs for some of these root parameters, especially with QTLs for total and specific RL, while another QTL for P accumulation in the HP environment on LG B2 was not associated with any of the root trait QTLs. Additional QTLs for P accumulation may be explained by basal root gravitropism, resulting in a shallower root system that may enhance root exploration of the surface soil horizons where P is concentrated (Bonser et al., 1996; Liao et al., 1999, 2004) or root hair and root exudate traits that also play an important role in P accumulation (Hinsinger 2001; Holford 1997; Yan et al., 2004). Apart from an understanding of physiological mechanisms of LP tolerance, the molecular markers identified in this study could be useful potential tools for marker assisted selection in breeding programs to select indirectly for these root traits that are difficult to evaluate in large populations. In this regard, this study will allow the pyramiding of root trait QTLs and especially the two P accumulation QTLs into a single genetic background through introgression breeding.

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REFERENCES

Barber, S.A. 1995 Soil nutrient bioavailability: A mechanistic approach. 2nd ed. John Wiley & Sons, Hoboken, NJ.

Basten, C.J., B.S. Weir, and Z.B. Zeng. 2005. QTL Cartographer. v. 1.15. Available at http://statgen.ncsu.edu/qtlcart/index.php [verified 8 Sept. 2005]. Dep. of Statistics, North Carolina State Univ., Raleigh.

Bates, T.R., and J.P. Lynch. 1996. Stimulation of root hair elongation in *Arabidopsis thaliana* by low phosphorus availability. Plant Cell Environ. 19:529–538.

Beebe, S., J. Lynch, N. Galwey, J. Tohme, and I. Ochoa. 1997. A geographical approach to identify phosphorus-efficient genotypes among landraces and wild ancestors of common bean. Euphytica 95:325–336.

Beebe, S., F. Pedraza, M. Rojas, J. Gutierrez, and J. Tohme. 1998. A genetic map of common bean combining RFLP, RAPD, SCAR and AFLP markers. Annu. Rep. Bean Improv. Coop. 41:95–96.

Blair, M.W., F. Pedraza, H.F. Buendia, E. Gaitán-Solís, S.E. Beebe, P. Gepts, and J. Tohme. 2003. Development of a genome-wide anchored microsatellite map for common bean (*Phaseolus vulgaris* L.) Theor. Appl. Genet. 107:1362–1374.

Bonser, A.M., J.P. Lynch, and S. Snapp. 1996. Effect of phosphorus

deficiency on growth angle of basal roots of *Phaseolus vulgaris* L. New Phytol. 132:281–288.
Borch, K., T.J. Bouma, J.P. Lynch, and K.M. Brown. 1999. Ethylene: A regulator of root architectural responses to soil phosphorus availability. Plant Cell Environ. 22:425–431.
Champoux, M.C., G. Wang, S. Sarkarung, D.J. Mackill, J.C. O'Toole, N. Huang, and S.R. McCouch. 1995. Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor. Appl. Genet. 90:969–981.
Churchill, G.A., and R.W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. Genetics 138:963–971.
CIAT. 1991. Bean program annual report. CIAT, Cali, Colombia.
CIAT. 1992. Constraints to and opportunities for improving bean production. A planning document 1993–1998. An achievement document 1987–1992. CIAT, Cali, Colombia.
CIAT. 1996. Bean program annual report. CIAT, Cali, Colombia.
Dellaporta, S.L., J. Wood, and J.B. Hicks. 1983. A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep. 1:19–21.
de Willigen, P., M. van Noordwijk. 1987. Roots, plant production and nutrient use efficiency. Ph.D. thesis. Wageningen Agricultural Univ., Wageningen, the Netherlands.
Eissenstat, D.M. 1992. Costs and benefits of constructing roots of small diameter. J. Plant Nutr. 15:763–782.
Fairhust, T., R. Lefroy, E. Mutert, and N. Batjes. 1999. The importance, distribution and causes of phosphorus deficiency as a constraint to crop production in the tropics. Agroforestry Forum 9:2–8.
Fan, M.S., J.M. Zhu, C. Richards, K.M. Brown, and J.P. Lynch. 2003. Physiological roles for aerenchyma in phosphorus-stressed roots. Funct. Plant Biol. 30:493–506.
Fisher, M.C.T., D.M. Eissenstat, and J.P. Lynch. 2002. Lack of evidence for programmed root senescence in common bean (*Phaseolus vulgaris*) grown at different levels of phosphorus supply. New Phytol. 153:63–71.
Fitter, A.H. 1985. Functional significance of root morphology and root system architecture. p. 87–106. *In* A.H. Fitter et al. (ed.) Ecological interactions in soil. Blackwell Scientific, Oxford, UK.
Freyre, R., P. Skroch, A.-F. Adam-Blondon, V. Geffry, A. Shirmohamadali, W.C. Johnson, V. Llaca, R.O. Nodari, P.A. Pereira, S.-M. Tsai, J. Tohme, M. Dron, J. Nienhuis, and P. Gepts. 1998. Towards an integrated linkage map of common bean. IV. Correlation among RFLP maps. Theor. Appl. Genet. 97:847–856.
Gaume, A., F. Mächler, C. De Leon, L. Narro, and E. Frossard. 2001. Low-P tolerance by maize (*Zea mays* L.) genotypes: Significance of root growth, and organic acids and acid phosphatase root exudation. Plant Soil 228:253–264.
Gepts, P. 1988. A middle American and an Andean common bean gene pool. p. 375–390. *In* P. Gepts (ed.) Genetic resources of *Phaseolus* beans. Kluwer Academic Publ., Dordrecht, the Netherlands.
Gu, W.K., N.F. Weeden, J. Yu, and D.H. Wallace. 1995. Large scale, cost-effective screening of PCR products in marker-assisted selection applications. Theor. Appl. Genet. 91:465–470.
Hinsinger, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: A review. Plant Soil 237:173–195.
Holford. I.C.R. 1997. Soil phosphorus: Its measurements and its uptake by plants. Aust. J. Soil Res. 35:227–239.
Kelly, J.D., P. Gepts, P.M. Miklas, and D.P. Coyne. 2003. Tagging and mapping of genes and QTL and molecular maker-assisted selection for traits of economic importance in bean and cowpea. Field Crops Res. 82:135–154.
Lander, E., P. Green, J. Abrahamson, A. Barlow, M. Daley, S. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental natural populations. Genomics 1:174–181.
Liao, H., G. Rubio, X.L. Yan, A.Q. Cao, K.M. Brown, and J.P. Lynch. 2001. Effect of phosphorus availability on basal root shallowness in common bean. Plant Soil 232:69–79.
Liao, H., G. Rubio, X. Yan, and J.P. Lynch. 1999. Gravitropic response of bean root system to phosphorus deficiency. p. 329–331. *In* J.P. Lynch and J. Deikman (ed.) Phosphorus in plant biology: Regulatory roles in molecular, cellular, organismic, and ecosystem processes. Am. Soc. Plant Physiol., Rockville, MD.
Liao, H., X. Yan, G. Rubio, S.E. Beebe, M.W. Blair, and J.P. Lynch. 2004. Genetic mapping of basal root gravitropism and phosphorus acquisition efficiency in common bean. Funct. Plant Biol. 31:959–970.
Lynch, J. 1995. Root architecture and plant productivity. Plant Physiol. 109:7–13.
Lynch, J., and S. Beebe. 1995. Adaptation of beans (*Phaseolus vulgaris* L.) to low phosphorus availability. Hortic. Sci. 30:1165–1171.
Lynch. J.P., and K.M. Brown. 1999. Regulation of root architecture by phosphorus availability. p. 148–156. *In* J.P. Lynch and J. Deikman (ed.) Phosphorus in plant biology: Regulatory roles in molecular, cellular, organismic, and ecosystem processes. Am. Soc. Plant Physiol., Rockville, USA.
Lynch, J.P., and K.M. Brown. 2001. Topsoil foraging—An architectural adaptation of plants to low phosphorus availability. Plant Soil 237:225–237.
Lynch, J., and M. Ho. 2004. Rhizoeconomics: Carbon costs of phosphorus acquisition. Plant Soil. 269:45–56.
Lynch, J., and J.J. van Beem. 1993. Growth and architecture of seedling roots of common bean genotypes. Crop Sci. 33:1253–1257.
Ma, Z., D.G. Bielenberg, K.M. Brown, and J.P. Lynch. 2001a. Regulation of root hair density by phosphorus availability in *Arabidopsis thaliana*. Plant Cell Environ. 24:459–467.
Ma, Z., T.C. Walk, A. Marcus, and J.P. Lynch. 2001b. Morphological synergism in root hair length, density, initiation and geometry for phosphorus acquisition in *Arabidopsis thaliana*: A modeling approach. Plant Soil 236:221–235.
Marschner, H. 1995. Mineral nutrition of higher plants. 2nd ed. Academic Press, London.
Miller, C.R., I. Ochoa, K.L. Nielsen, D. Beck, and J.P. Lynch. 2003. Genetic variation for adventitious rooting in response to low phosphorus availability: Potential utility for phosphorus acquisition from stratified soils. Funct. Plant Biol. 30:973–985.
Murphy, J., and J. Riley. 1963. A modified single solution method for the determination of phosphate in natural waters. Anal. Chem. Acta 27:31–35.
Nielsen, K.L., A. Eshel, and J.P. Lynch. 2001. The effect of phosphorus availability on the carbon economy of contrasting common bean (*Phaseolus vulgaris* L.) genotypes. J. Exp. Bot. 52:329–339.
Nodari, R.O., E.M.K. Koinange, J.D. Kelly, and P. Gepts. 1992. Towards an integrated linkage map of common bean. I. Development of genomic DNA probes and levels of restriction fragment length polymorphism. Theor. Appl. Genet. 84:186–192.
Nodari, R.O., S.M. Tsai, R.L. Gilbertson, and P. Gepts. 1993. Towards an integrated linkage map of common bean. II. Development of an RFLP-based linkage map. Theor. Appl. Genet. 85:513–520.
Price, A.H., and A.D. Tomos. 1997. Genetic dissection of root growth in rice (*Oryza sativa* L.): II. Mapping quantitative trait loci using molecular markers. Theor. Appl. Genet. 95:143–152.
Price, A.H., A.D. Tomos, and D.S. Virk. 1997. Genetic dissection of root growth in rice (*Oryza sativa* L.): I. A hydroponic screen. Theor. Appl. Genet. 95:132–142.
Rachier, G.O., C.S. Wortmann, and J.S. Tenywa. 1998. Low phosphorus tolerance in common bean as affected by root architecture. Bean Improv. Coop. Ann. Rep. 41:206–208.
Raghothama, K. 1999. Phosphate acquisition. Ann. Rev. Plant Physiol. Plant Mol. Biol. 50:665–693.
Rao, I.M. 2001. Role of physiology in improving crop adaptation to abiotic stresses in the tropics: The case of common bean and tropical forages. p. 583–613. *In* M. Pessarakli (ed.) Handbook of plant and crop physiology. Marcel Dekker, New York.
Rao, I.M., D.K. Friesen, and M. Osaki. 1999. Plant adaptation to phosphorus-limited tropical soils. p. 61–96. *In* M. Pessarakli (ed.) Handbook of plant and crop stress. Marcel Dekker, New York.
Ryser, P. 1996. The importance of tissue density for growth and life span of leaves and roots: A comparison of five ecologically contrasting grasses. Funct. Ecol. 10:717–723.
SAS Institute. 1987. SAS user's guide: Statistics. 6th ed. SAS Inst., Cary, NC.
Sattelmacher, B., W.J. Horst, and H.C. Becker. 1994. Factors that contribute to genetic variation for nutrient efficiency of crop plants. Z. Pflanzenernaehr. Bodenkd. 157:215–224.
Schneider, K.A., M.E. Brothers, and J.D. Kelly. 1997. Marker assisted selection to improve drought resistance in common bean. Crop Sci. 37:51–60.

Schoonhoven, A. van, and M.P. Pastor-Corrales. 1987. Standard system for the evaluation of bean germplasm. CIAT, Cali, Colombia.
Singh, S.P., P. Gepts, and D.G. Debouck. 1991. Races of common bean (*Phaseolus vulgaris* Fabacea). Econ. Bot. 45:379–396.
Thung, M. 1991. Bean agronomy in monoculture. p. 737–835. *In* A. van Schoonhoven and O. Voysest (ed.) Common beans: Research for crop improvement. CAB International and CIAT, Wallingford, UK.
Tohme, J., D.O. Gonzalez, S. Beebe, and M.C. Duque. 1996. AFLP analysis of gene pools of a wild bean core collection. Crop Sci. 36: 1375–1384.
Vallejos, E.C., N.S. Sakiyama, and C.D. Chase. 1992. A molecular marker-based linkage map of *Phaseolus vulgaris* L. Genetics 131: 733–740.
Vance, C.P. 2001. Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. Plant Physiol. 127:390–397.
Wissuwa, M., and N. Ae. 2001. Further characterization of two QTLs that increase phosphorus uptake of rice (*Oryza sativa* L.) under phosphorus deficiency. Plant Soil 237:275–286.
Wissuwa, M., J. Wegner, N. Ae, and M. Yano. 2002. Substitution mapping of Pup1: A major QTL increasing phosphorus uptake of rice from a phosphorus-deficient soil. Theor. Appl. Genet. 105: 890–897.
Wissuwa, M., Yano, M., Ae, N. 1998. Mapping of QTLs for phosphorus-deficiency tolerance in rice (*Oryza sativa* L.). Theor. Appl. Genet. 97:777–783.
Yan, X, S. Beebe, J.P. Lynch. 1995a. Genetic variation for phosphorus efficiency of common bean in contrasting soil types: II. Yield response. Crop Sci. 35:1094–1099.
Yan, X., H. Liao, S. Beebe, M. Blair, and J. Lynch. 2004. QTL mapping of root hair and acid exudation traits and their relationship to phosphorus uptake in common bean. Plant Soil 265:17–29.
Yan, X, J.P. Lynch, and S. Beebe. 1995b. Genetic variation for phosphorus efficiency of common bean in contrasting soil types: I. Vegetative response. Crop Sci. 35:1086–1093.
Youngdahl, L.J. 1990. Differences in phosphorus efficiency in bean genotypes. J. Plant Nutr. 13:1381–1392.
Yu, K, S.J. Park, V. Poysa, and P. Gepts. 2000. Integration of simple sequence repeat (SSR) markers into a molecular linkage map of common bean (*Phaseolus vulgaris* L.). J. Hered. 91:429–434.
Zhu, J., S. Kaeppler, and J.P. Lynch. 2004. Mapping of QTL controlling root hair length in maize (*Zea mays* L.) under phosphorus deficiency. Plant Soil. 265:17–29.