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Genetic mapping of basal root gravitropism and phosphorus acquisition efficiency in common bean

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Abstract. Root gravitropism determines the relative distribution of plant roots in different soil layers, and therefore, may influence the acquisition of shallow soil resources such as phosphorus (P). Growth pouch and field studies were conducted to evaluate root gravitropism of common bean (*Phaseolus vulgaris* L.) in response to P deficiency and to detect quantitative trait loci (QTL) associated with this trait. A deep-rooted genotype, DOR364, was crossed with a shallow-rooted genotype, G19833, to obtain 86 $F_{5:7}$ recombinant inbred lines (RILs). Root gravitropic traits were measured as basal root growth angle (BRGA), shallow basal root length (SBRL, basal root length in the top 0–3 cm of soil) and relative shallow basal root length (RSBRL, percentage of basal root length in the top 0–3 cm of soil relative to total basal root length). Large genetic variability for these traits was found in the parents and RILs, with BRGA ranging from –18.73 to 56.69° and SBRL ranging from 0.42 to 2.63 m per plant. The parents and six RILs with contrasting root gravitropism were further evaluated in the field, where root shallowness was significantly correlated with plant growth and P uptake. QTL were detected by single point analysis (SPA), interval mapping (IM) and composite interval mapping (CIM) techniques with a genetic map for the DOR364 × G19833 population consisting of 236 molecular markers. The IM / CIM QTL were detected among the 11 linkage groups of common bean, with 16 QTL controlling the above root traits and six QTL controlling P acquisition efficiency (PAE) in the field study. At least three of the root trait QTL were associated with QTL for PAE, suggesting that root gravitropic traits are associated with PAE and that QTL for these traits can be used to facilitate selection and breeding for higher P efficiency in common bean and other crops.

Keywords: basal root growth angle, common bean, phosphorus acquisition efficiency, root gravitropism.

Introduction

Common bean (*Phaseolus vulgaris*) is the most important food legume on earth, providing protein for over 500 million people. As a vegetable, it provides vitamin A, iron (Fe) and other scarce nutrients to over a billion people in developing countries (FAO 1991). Common bean is often grown in low input farming systems, where soil nutrient deficiency is commonly encountered, but where fertilisers are not sufficiently available or affordable. Among the mineral stresses, P deficiency is the primary constraint to bean production in the tropics and subtropics, limiting yield in at least 60% of the bean-producing areas of Latin America and Africa (Beebe *et al.* 1997; Wortmann *et al.* 1998). The existence of genetic variation for P efficiency, defined as the ability to grow or yield at low P availability, offers the possibility to develop bean genotypes with superior adaptation to low P soils (Lynch 1998*a*; Vance *et al.* 2003).

Abbreviations used: AFLP, amplified fragment length polymorphism; BRGA, basal root growth angle; CIM, composite interval mapping; IM, interval mapping; LOD, logarithm of odds; LR, likelihood ratio; PAE, phosphorus acquisition efficiency; QTL, quantitative trait loci; RAPD, randomly amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; RILs, recombinant inbred lines; RSBRL, relative shallow basal root length; SBRL, shallow basal root length; SDW, shoot dry weight; SPA, single point analysis.

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It has been demonstrated in both field and greenhouse studies that there is substantial genetic variation in P efficiency in bean (Gerloff and Gabelman 1983; Thung 1990; CIAT 1991). Promising genotypes for low P tolerance have been identified in contrasting gene pools and races of common bean (Yan *et al.* 1995*a*, *b*). These genotypes are very diverse with respect to agronomic traits and evolutionary origins, suggesting that they may possess diverse genes and mechanisms that can be recombined for improved tolerance (Beebe *et al.* 1997). However, attempts to improve P efficiency through simple selection have not been very successful owing to the confounding effects of other environmental factors on response to P stress *per se* (Singh *et al.* 1989). In fact, interactions of genotype × season, genotype × P level, as well as genotype × season × P level, are common (Lynch and Beebe 1995). These interactions underscore the difficulty in relying on yield performance as a sole criterion of selection for a breeding program. Yield trials with environmental stress are costly and suffer from spatial variability, and hence, identification of the specific mechanism(s) of phosphorus efficiency would be more reliable. If a complex character such as P efficiency could be resolved into physiological traits governed by discrete mechanisms, it may be more reliable to tag the traits with molecular markers than to measure P efficiency as a quantitative trait by yield trials (Lynch 1998*b*).

Previous studies have indicated that root gravitropic response to P deficiency may be one of the mechanisms of P efficiency in bean. Under low P conditions, some bean genotypes have less basal root gravitropism, resulting in a shallower root system (Bonser *et al.* 1996; Liao *et al.* 2001). Basal roots are a distinct class of root arising near the basal end of the hypocotyl (Zobel 1996), which form the structural scaffold upon which the majority of the bean root system develops. A shallow root system may enhance root exploration of the surface soil horizons, where P availability is greatest in most soils (Chu and Chang 1966; Pothuluri *et al.* 1986). Geometric simulation modelling also suggests that genotypes with shallow basal roots are more efficient in P acquisition than genotypes with deeper basal roots, especially in stratified soils with heterogeneous P distribution (Ge *et al.* 2000). Therefore, root gravitropism is a potentially beneficial trait for P efficiency (Lynch 1998*b*; Lynch and Brown 2001).

Root gravitropism is relatively difficult to observe and quantify under field conditions in a nondestructive manner and is subject to environmental plasticity. In previous studies we developed a growth pouch system that permits the analysis of root geometry in two dimensions, including the distribution of roots in different layers and the measurement of root growth angles. The parameters measured in the growth pouch system were highly correlated with those measured in sand and soil pot culture (Bonser *et al.* 1996; Liao *et al.* 2001). Due to its relative simplicity, this system is suited to large experiments, such as those required for genetic mapping.

Root gravitropism in common bean is thought to be a quantitative trait amenable to molecular marker analysis to identify and map QTL, as demonstrated for root traits in other crops (Champoux *et al.*1995; Price and Tomos 1997; Price *et al.* 1997; Ni *et al.* 1998; Hu *et al.* 2001; Wissuwa and Ae 2001; Zhang *et al.* 2001; Wissuwa *et al.* 2002). Molecular markers linked to important root QTL would be useful as selection criteria for field breeding work: a process known as marker-assisted selection (Jones *et al.* 1997). Therefore, the objective of the present study was to identify QTL for three root gravitropism traits, namely, basal root growth angle, shallow basal root length, and relative shallow basal root length, and to evaluate their correlation with plant P efficiency, as a step towards improving the adaptation of cultivars of common bean to low P soils.

Materials and methods

Plant materials

This study included two parental genotypes of common bean (*Phaseolus vulgaris* L.), DOR364 and G19833, and 86 $F_{5.7}$ derived RILs. DOR364 belongs to race M of the Mesoamerican gene pool, and G19833 belongs to race Nueva Granada of the Andean gene pool (Singh *et al.* 1991). DOR364 is a high yielding cultivar developed in Central America that yields poorly under P-deficient conditions, while G19833 is a landrace from Peru that is relatively well adapted to P-limited conditions, yielding nearly twice that of check varieties under severe P stress (Lynch and Beebe 1995; Yan *et al.* 1995*a*, *b*; Beebe *et al.* 1997). The two parents also have been characterised as contrasting in root architecture under low P conditions; G19833 has a shallower root system than DOR364 (Bonser *et al.* 1996; Liao *et al.* 2001). These two genotypes were crossed and the progenies advanced by the single seed descent (SSD) method to the F_5 generation, then with bulk harvesting the F_5 derived lines were advanced to the F_7 generation (these materials are called $F_{5.7}$ RILs) (Beebe *et al.* 1998).

Growth pouches

The parents and RILs were grown in a growth pouch system as described previously (Bonser *et al.* 1996; Liao *et al.* 2001). The growth pouch consisted of a 24.1 × 30.5 cm sheet of phosphorus-free blue germination paper (Anchor Paper Co., St Paul, MN) and a polyethylene bag that was punctured evenly (2 × 2 cm) with small holes to improve aeration. The pouches were placed upright into 30 × 20.8 × 30.9 cm plastic containers each having 2 L of nutrient solution composed of (in μM) 3000 KNO_3, 2000 $Ca(NO_3)_2$, 250 $MgSO_4$, 25 KCl, 12.5 H_3BO_3, 1 $MnSO_4$, 1 $ZnSO_4$, 0.25 $CuSO_4$, 0.25 $(NH_4)_6Mo_7O_{24}$, and 25 Fe-Na-EDTA. The parental genotypes and 86 RILs were treated with low phosphorus [0.2 μM $NH_4H_2PO_4$ plus 500 μM $(NH_4)_2SO_4$] or high phosphorus (1000 μM $NH_4H_2PO_4$).

Sterilised seeds of the two parental genotypes and the 86 RILs with cotyledons were germinated over 48 h at 25°C on paper towels moistened with 0.5 mM $CaSO_4$. Germinated seeds with an emerging radical 2–3 cm in length were transferred to growth pouches. Plants in growth pouches were placed in a growth chamber at 400 μmol photons m^{-2} s^{-1} of photosynthetically active radiation for 12 h at 25°C, alternated with a 12 h dark period at 20°C, relative humidity 60 / 85% (day / night). Plants were grown and harvested in randomised blocks in four replicates (each replicate included one plant). At 6 d after transplanting, intact

root systems on the germination paper were scanned into a computer as digital images. The root system was divided into two horizontal layers (0–3 and 3–30 cm) in Adobe Photoshop 5.0 (Adobe System Inc., San Jose, CA), then the basal root length in each layer was measured with root image analysis software (WinRhizo Pro, Régent Instruments, Québec, Canada). SBRL was measured as basal root length in the top 0–3 cm of the pouch. RBRL was calculated as the percentage of basal root length in the top 0–3 cm of the pouch relative to total basal root length of a plant. BRGA was determined as the tangent formed by the vertical height of basal roots over 2 cm horizontal length (Liao *et al.* 2001). Root growth angles were averaged over all basal roots in a plant. A negative growth angle means the root is growing upward.

Field study of the RIL population

The field trial was conducted in Darién, Colombia (3°55′60″N, 76°31′0″W; 1450 m above sea level; 20°C average yearly temperature; andisol soil type). The native soil P at this site was 2 mg kg^{-1} as determined by the Bray II extraction method (Bray and Kurtz 1945). Triple superphosphate was broadcast and incorporated in the soil at the rate of 6 kg P ha^{-1} 6 months prior to the experiment. Parental genotypes were planted with six replicates and 71 RILs were planted with three replicates in a randomised complete block design. Ten seeds were sown per experimental plot in single rows with 1.0 × 0.6 m spacing. At flowering (40 d after planting), plants were harvested to determine plant dry weight and total P uptake.

Field study of selected lines

The two parents and six extreme RILs with cotyledons in growth pouches, three having shallow root systems and three having deep root systems, were selected for another field study in a low P soil in south China. Seeds were sterilised and germinated as described above, then transplanted at the field site. The field site was located in Guangzhou, China (26°06′N, 113°15′E, 25–35°C average daily temperature, ultisol soil type). The soil was a sandy loam with 520 mg kg^{-1} of total P, yet only 17.76 mg kg^{-1} of available P in the top 0–20 cm of the soil profile and 2.56 mg kg^{-1} of available P at depths below 20 cm, as determined by Bray II extraction. Soil pH was adjusted to 5.8 with lime (1500 kg ha^{-1}) 2 weeks before fertilisation. There were two P treatments: a high P treatment in which soil was fertilized with 160 kg P ha^{-1} soil as triple superphosphate (TSP), and a low P treatment in which soil was not fertilised with P. Both treatments received 180 kg N ha^{-1} as urea and 200 kg K ha^{-1} as KCl. The field site was divided into five blocks, each containing a completely randomised arrangement of two phosphorus treatments with eight genotypes. Four seeds were sown per single row plot 5 m in length and at 1 m spacing between rows. Conventional field practices including irrigation and pest and disease management were adopted in this field trial. Plant shoots were harvested 15 d after transplanting and dry weights were recorded after 3 d of drying in an oven at 75°C. Tissue P content was measured spectrophotometrically (Murphy and Riley 1962).

Root distribution in the soil profile was analysed following a modified version of the profile wall method described by Schuurman and Goedewaagen (1971). Tangential trenches were dug at 5 cm from the row of plant stems (shoot bases). The walls were carefully scraped with a screwdriver to reveal the tips of the roots. Plastic transparent sheets (21.5 × 27.9 cm) were positioned adjacent to the exposed soil wall on the side of the trench toward the nearest plant. The tips of the cut roots were mapped with a marker on the sheets, and the different root categories were distinguished by colors. According to the intersection of basal roots with the transparent sheet, the BRGA as determined from measurements in the field was calculated as follows and averaged over all basal roots that intersected the plastic sheet (Fig. 1):

$$\mathrm{Tan(BRGA)} = \mathrm{VD} / \sqrt{(\mathrm{HD} \times \mathrm{HD} + 5 \times 5)},$$

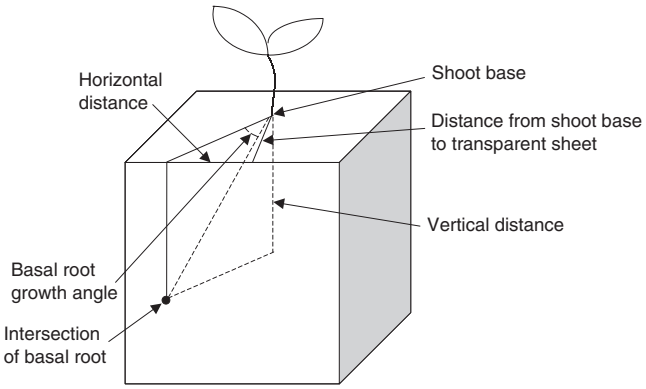


Fig. 1. Root angle measurement in the field. The intersection of a single basal root with the plastic sheet is indicated, •. The angle of the line passing through that circle and the base of the shoot, as measured from the horizontal, is a BRGA. When the angles of all such basal roots on the plant that intersect the plastic sheet have been measured and averaged, the resulting statistic is the BRGA for that plant. The measurement labeled 'horizontal distance' is the distance between the root–sheet intersection and the vertical plane that passes through the shoot base and that is perpendicular to the plastic sheet. The distance from the shoot base to the plastic sheet was always 5 cm.

where Tan is tangent, VD is vertical distance of root intersection from hypocotyl base, HD is the distance between the root–sheet intersection and the vertical plane that passes through the shoot base and that is perpendicular to the plastic sheet. The distance of the shoot base to transparent plastic sheet was always 5 cm.

Statistical analysis

Where statistical treatments were appropriate, the data were analysed by analysis of variance (ANOVA) and means were compared by post-hoc pairwise contrasts in SYSTAT 5.2.1 (Wilkinson *et al.* 1992). Regression statistics were obtained from Pearson linear correlation, which was used to analyse the relationship between root gravitropic traits and P uptake.

Molecular mapping of QTL associated with root gravitropic traits and phosphorus uptake

DNA was extracted from parental genotypes by the method of Dellaporta *et al.* (1983) with some modifications. Experimental procedures for the mapping of 50 restriction fragment length polymorphism (RFLP) probes and 24 amplified fragment length polymorphism (AFLP) markers were as described by Beebe *et al.* (1998), while amplification conditions for 32 microsatellite markers were as described by Blair *et al.* (2003). In addition, sequence characterised 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 (Operon Biotechnologies GmbH, Nattermannallee, Germany) were used to generate 124 randomly amplified polymorphic DNA (RAPD) markers. A total of 236 markers were used to create a complete genetic map, extending the map that was reported previously (Beebe *et al.* 1998), with the Mapmaker program (Lander *et al.* 1987). Linkage analysis was conducted initially for the RFLP and microsatellite markers at logarithm of odds (LOD) six to anchor the map to the core map of Freyre *et al.* (1998). Remaining markers were placed individually at a LOD > 3.5 with the 'assign' and 'place' functions and confirmed by the 'ripple' function in Mapmaker. Putative QTL were detected by SPA, IM and CIM with the software program QTL Cartographer 2.0 (Basten *et al.* 1999). Single point analysis was used to identify specific markers

associated with the phenotypic traits that could be used in future marker-assisted selection, while interval mapping was used to identify the most probable location of the QTL itself. Probability thresholds of 0.05, 0.01 and 0.001 were used for SPA. Parameters for IM and CIM analysis included a forward / backward regression with a window size of 10 cM, a walkspeed of 1 cM, five background markers and probability thresholds of 0.05 each for the partial *F*-test for both marker inclusion and exclusion. Traits analysed included: BRGA, SBRL, RSBRL, and PAE in the field. The LOD threshold was 2.0 for QTL detected for PAE and 2.5 for all other traits in the IM and CIM analysis, and analyses were conducted with both entry mean and single repetition data sets.

Results

Plants in the field trial in Colombia expressed P deficiency symptoms as expected, given the low level of phosphorus fertilisation utilised. A nearby trial with a much higher level of P application displayed far better plant development, confirming that P was severely limiting at the study site. For present purposes P acquisition data from this trial and the respective QTL identified will be utilised as a baseline for comparison to other data reported here, while an extensive description of the field results *per se* will be reported elsewhere.

The three root gravitropic traits measured (i.e. BRGA, SBRL, and RSBRL) varied significantly between the two parents and among the RILs (Table 1). At low P, the shallow-rooted genotype G19833 had significantly greater SBRL, greater RSBRL and smaller BRGAs than DOR364. However, under high P conditions, the two parents did not differ significantly in these traits (Table 1), indicating that the parents have contrasting gravitropic responses to P deficiency. BRGA in G19833 significantly differed between low and high P, but not in DOR364 (Table 1). Significant genetic variation for root gravitropic traits also was observed among the RILs in low P, with the means of BRGAs ranging from –18.73 to 56.69° and SBRL ranging from 0.42 to 2.63 m per plant (Table 1; Fig. 2). The root gravitropic traits segregated and displayed a normal distribution in the $F_{5.7}$ population, suggesting a quantitative inheritance of these traits (Fig. 2). The mean values calculated across all of the RILs for each of the three root gravitropic traits were skewed toward the shallow-rooted parent, G19833 (Table 1; Fig. 2). There was substantial transgressive segregation in the $F_{5.7}$ progenies, with nearly 10% of progenies having BRGA lower than that of G19833, 10% of progenies having greater SBRL than G19833, and 30% of progenies having greater relative SBRL than G19833. In contrast, there were a few progenies with larger BRGA, less SBRL and RSBRL than DOR364 (Fig. 2). Under high P conditions, the RILs also showed significant segregation for the three traits, but the variance was much smaller than that in low P, again indicating some plasticity of these traits in response to high *v.* low P (Table 1).

To evaluate the relationship between root gravitropic traits and PAE, the parents and six selected RILs were grown in the field in south China where P deficiency is a primary constraint to plant growth. Phosphorus significantly affected plant growth of the parental genotypes and the RILs (Tables 2, 3). Plant biomass and P uptake increased with increasing P level (Table 2). The shallow-rooted G19833 outperformed the deep-rooted DOR364 in both plant biomass and P uptake at low P, but the difference diminished at high P. The RILs also differed in plant biomass and P content. In general, RILs with shallow root systems (RILs 33, 39 and 87) had higher plant biomass and P uptake than RILs in low P, but the difference diminished under high P conditions. When we combined G19833 and RILs with shallow root systems as a shallow-rooting group and DOR364 and RILs with deep root systems

Table 1. Mean, *F*-value and significance level from ANOVA of root gravitropic traits in parents and recombinant inbred lines (RILs) of common bean due to genotype effect in growth pouches at low phosphorus (0.2 μM P) and high phosphorus (1000 μM P)

The number of RILs is 86 and 6 in low and high phosphorus, respectively. BRGA (degrees from horizontal); SBRL (m plant^{-1} in the top 1–3 cm); RSBRL (calculated as the percentage of basal root length in a given layer relative to total basal root length of a plant). The *F*-value for ANOVA of P effect on BRGA in G19833 is 47.99, significant to 0.00044. *, $0.05>P>0.01$; **, $0.01>P>0.001$; ***, $P<0.001$; ns, not significant

Root gravitropic trait	Parent genotype DOR364	Parent genotype G19833	*F*-value	RILs Mean	RILs Range	*F*-value
Low P						
BRGA	35.85 ± 1.64	2.08 ± 1.81	203.865***	12.00 ± 0.65	−18.73–56.69	2.20***
SBRL	0.82 ± 0.03	1.36 ± 0.10	20.932**	1.06 ± 0.02	0.42–2.63	2.14***
RSBRL	44.82 ± 2.98	61.76 ± 3.00	12.472*	58.75 ± 0.69	21.84–95.21	1.55**
High P						
BRGA	36.64 ± 6.59	23.63 ± 1.74	3.642 ns	13.1 ± 2.54	−9.63–47.58	2.69*
SBRL	0.98 ± 0.07	1.22 ± 0.10	3.636 ns	1.20 ± 0.09	0.61–3.09	2.67*
RSBRL	46.63 ± 2.16	54.09 ± 3.35	3.498 ns	63.16 ± 2.17	33.65–92.45	4.77**

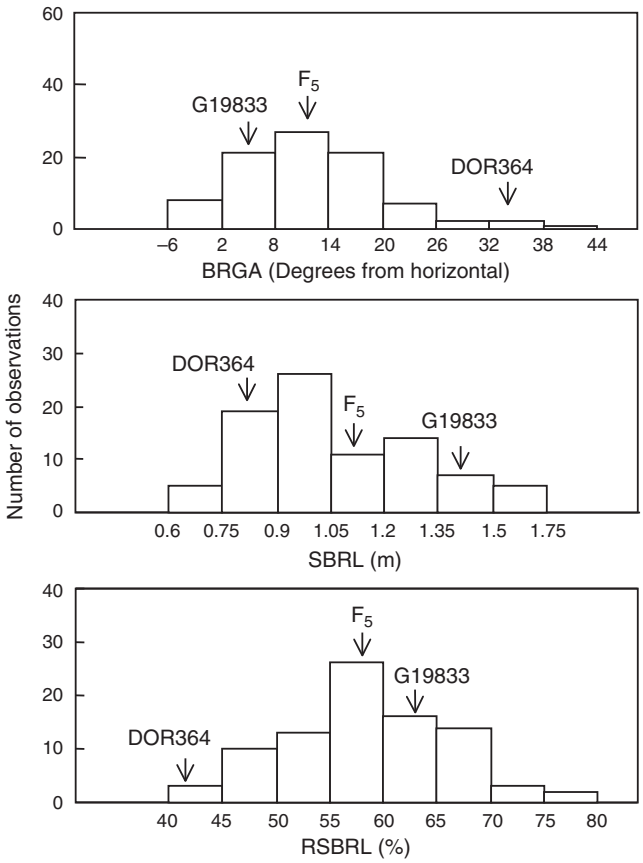


Fig. 2. Frequency distribution of three root gravitropic traits among the RILs grown in pouches. BRGA (degrees from horizontal); SBRL (m plant^{-1}); RSBRL (calculated as the percentage of basal root length in a given layer relative to total basal root length of a plant). Arrows indicate value of parents and mean value of RILs.

as a deep-rooting group to perform group analysis, we found that RILs with shallow root systems had significantly higher shoot biomass and P uptake than those with deep root systems under low P but not under high P conditions (Tables 2, 3).

Phosphorus supply also significantly affected the BRGA of parents and RILs in the field (Tables 2, 3). The parents contrasted in BRGA in response to phosphorus deficiency in the field. Increases in the P supply increased the BRGA of the shallow-rooted parent, G19833, while the deep-rooted parent, DOR364 was not affected by the P treatments (Table 2). There was plasticity of BRGA in response to P deficiency in the shallow-rooted parent, as indicated by the consistently higher BRGA in the high P treatments compared with the low P treatments.

Shoot biomass, plant P uptake and BRGA in the field were significantly correlated with BRGA, basal root length and relative basal root allocation to surface horizons in growth pouches at low P (Table 4). This indicates that 2-dimensional measurements 7 of root gravitropic traits in growth pouches may be good indicators of PAE in the field.

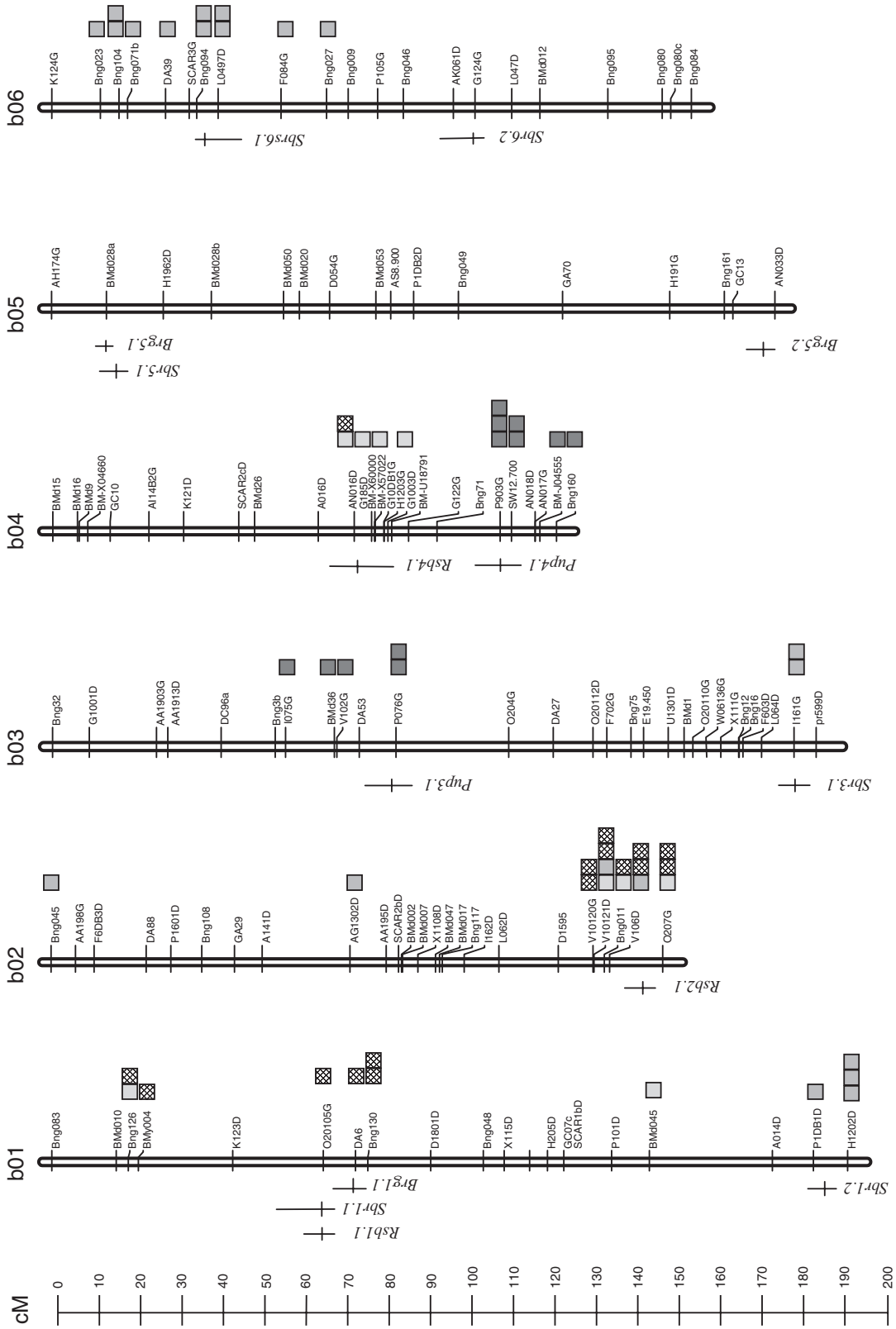
Genetic polymorphism between the DOR364 and G19833 parents was high with both RFLP and SSR markers facilitating the creation of an anchored framework map which was extended by the additional AFLP, RAPD and SCAR markers. The resulting map covered all 11 chromosomes of the *Phaseolus* genome and updated the map reported by Beebe *et al.* (1998). The order of the RFLP markers was the same as that represented by Vallejos *et al.* (1992) and could be correlated readily with the integrated map by Freyre *et al.* (1998), whose chromosome naming conventions are used and which allows cross-population comparisons of QTL locations.

Single point regression analysis was performed on all the marker × trait combinations to identify markers that were significantly associated (at P=0.05, 0.01 or 0.001 levels of significance) with each of the root characters or with the PAE trait across the 11 linkage groups of the genetic map. A total of six regions (defined as one or several adjacent markers showing association with a trait) on six separate linkage groups were involved in PAE in the field (Fig. 3). Meanwhile,

Table 2. Shoot biomass and phosphorus uptake of parental beans and 6 recombinant inbred lines (RILs) grown in the field for 15 d under low phosphorus (LP, no added P) and high phosphorus (HP, 160 kg P ha^{-1})

Each value is the mean of four replicates with standard error in parentheses

		Parental genotype							
	P	DOR364	G19833	RILs with deep root system			RILs with shallow root system		
	treatment	(deep)	(shallow)	32	38	70	33	39	87
Shoot biomass	LP	0.29 (0.05)	0.49 (0.06)	0.28 (0.04)	0.27 (0.03)	0.32 (0.02)	0.42 (0.06)	0.38 (0.05)	0.39 (0.04)
(g plant^{-1})	HP	0.60 (0.17)	0.62 (0.10)	0.64 (0.12)	0.49 (0.04)	0.63 (0.05)	0.52 (0.05)	0.46 (0.02)	0.51 (0.10)
P content	LP	0.54 (0.12)	1.27 (0.06)	0.57 (0.06)	0.49 (0.04)	0.67 (0.05)	0.93 (0.05)	0.85 (0.15)	0.83 (0.08)
(mg P plant^{-1})	HP	1.91 (0.60)	2.26 (0.49)	2.45 (0.57)	1.63 (0.09)	2.53 (0.40)	1.97 (0.13)	1.63 (0.12)	2.11 (0.42)
BRGA (degrees	LP	40.56 (3.89)	29.74 (1.34)	41.29 (1.78)	36.82 (4.14)	42.78 (3.46)	21.62 (6.53)	31.66 (4.51)	28.89 (2.58)
from horizontal)	HP	39.39 (4.82)	49.19 (13.02)	33.97 (6.22)	60.52 (14.72)	51.17 (12.11)	99.08 (15.51)	53.72 (18.15)	51.23 (12.55)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RsbI.4
b05
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D054G
BMd053
AS8.900
P1DB2D
Bng049
GA70
H191G
Bng161
GC13
AN033D
SbrI.5
BrgI.5
BrgI.2
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Bng023
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SbrI.6
SbrI.2

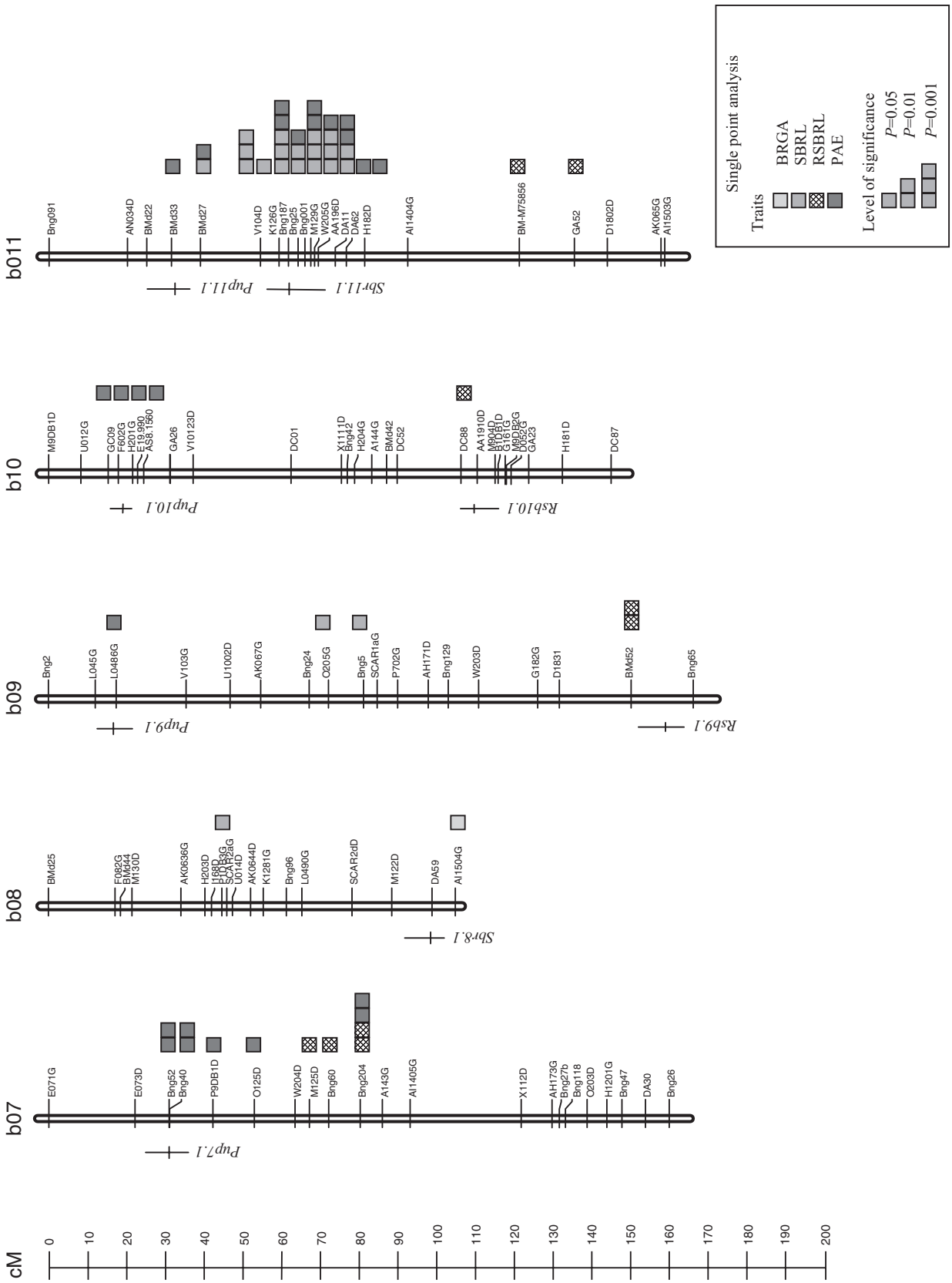

Fig. 3. Genetic map of common bean showing QTL conferring root gravitropic traits measured in growth pouch experiments and PAE measured in a field experiment as detected by SPA (squares) and interval mapping or composite interval mapping analysis (lines where length in the vertical axis represents the interval that is above LOD threshold and horizontal line represents position of peak LOD score). Chromosomes identified according to Freyre *et al.* (1998).

Table 3. *F*-values for ANOVA from an analysis of shoot dry weight, phosphorus uptake and BRGA in the field

The first three data columns show the whole-experiment analysis; the fourth and fifth columns show the low and high P treatments analysed separately. *, $0.05>P>0.01$; **, $0.01>P>0.001$; ***, $P<0.001$; ns, not significant at the 0.05 level. Shallowness shows G19833 and RILs with shallow root system as one group, DOR364 and RILs with deep root system as another group. BRGA, degrees from horizontal

Trait	Shallowness	P level	Shallowness × P	Effect of shallowness	
				Low P	High P
SDW	0.16 ns	29.83***	4.00*	8.55**	0.82 ns
P content	0.42 ns	33.84***	0.64 ns	9.23**	0.01 ns
BRGA	0.28 ns	9.60**	3.72*	16.12**	0.56 ns

Table 4. Correlation (Pearson linear correlation coefficient, *R*) between shoot biomass, P content or BRGA in south China field experiment and root gravitropic traits in growth pouches under phosphorus-deficient conditions

For growth pouches the number of observations was 8. BRGA, degrees from horizontal; SBRL, in the top 0–3 cm (m plant^{-1}); RSBRL in the top 0–3 cm, calculated as the percentage of basal root length in a given layer relative to total basal root length of a plant. *, $0.05>P>0.01$; **, $0.01>P>0.001$; ***, $P<0.001$

Study		Field			Growth pouches	
		SDW	P content	BRGA	SBRL	RSBRL
Growth pouches	BRGA	–0.86**	–0.84**	0.77*	–0.87**	–0.78*
	SBRL	0.82**	0.77*	–0.66*	1	0.68*
Field	RSBRL	0.67*	0.60	–0.81**	0.68*	1
	BRGA	–0.78*	–0.70*	1	–0.66*	–0.81*

over 20 regions from all 11 linkage groups had a significant effect on the three root gravitropic traits including: including four for BRGA, nine for SBRL and eight for RSBRL. Three of these loci on chromosome b04, b07 and b11 were closely linked to QTL conferring PAE in the field.

Interval mapping and composite interval mapping analysis confirmed many of the QTL found with SPA, especially those of higher significance (Fig. 3). IM / CIM analysis uncovered the same six QTL found for P uptake in the field and identified additional QTL for BRGA and for SBRL on chromosome b05 and b06 (*Brg*5.1, *Brg*5.2, *Sbr*5.1 and *Sbr*6.1) that had not been found with SPA (Table 5; Fig. 3). In addition, IM / CIM analysis uncovered a total of 16 QTL for gravitropic root traits. The determination coefficient (R^2) values for the PAE QTL ranged from 9.3 to 14.7%. The variance explained by QTL for the root gravitropism traits was higher on the other hand, reaching 15.9, 20.3 and 18.3% (R^2) for BRGA, SBRL and RSBRL traits, respectively.

Although the QTL for phosphorus uptake in the field on chromosomes b03, b09 and b10 (*Pup*3.1, *Pup*9.1 and *Pup*10.1) were not associated with QTL for other root traits, the QTL on chromosomes b04, b07 and b11 (*Pup*4.1, *Pup*7.1 and *Pup*11.1) were closely linked to IM / CIM QTL for RSBRL (*Rsb*4.1) and SBRL (*Sbr*11.1) as well as to SPA QTL for BRGA and RSBRL on chromosome b04, RSBRL on chromosome b07 and SBRL on chromosome b11. The above results indicate that QTL for root gravitropic traits at low P in growth pouches were closely linked in several cases with QTL for PAE in the field. This is consistent with the results from the field study above where the root gravitropic traits are significantly correlated with plant growth and P uptake under low P conditions. There were also co-localization of IM / CIM QTL for the three root gravitropism traits on chromosome b01 and the QTL for BRGA and RSBRL or QTL for BRGA and SBRL on chromosomes b02 and b05, respectively, indicating associations between the traits.

As expected, the positive allele for most QTL conferring shallow rooting gravitropic traits (10 out of 16) came from G19833 (Table 5), the parental genotype with a shallower root system. Interestingly the QTL for PAE derived from G19833 (*Pup*4.1, *Pup*7.1 and *Pup*11.1) were associated with shallower root traits, while the QTL for PAE derived from DOR364 (*Pup*3.1, *Pup*9.1 and *Pup*10.1) were not.

Discussion

The results from the present study confirm our previous observations that substantial genetic variability exists for

Table 5. Quantitative trait loci found for root gravitropic traits and P uptake in low P conditions in the field using interval mapping and composite interval mapping

BRGA, degrees from horizontal; SBRL, mm root length in the top 0–3 cm; RSBRL, percentage of root length in the top 0–3 cm; PAE (P uptake under low P), mg P plant^{-1}. LOD threshold was 2.0 for QTL detected for P uptake trait and 2.5 for all other traits

QTL name	Name of trait	QTL method	Chromosome	Closest marker	Marker interval	LR	LOD	R^2	Total R^2	Additivity	Source
Brg1.1	BRGA	CIM	1	DA60	O20105G–Bng130	16.4	3.6	14.0	35.4	4.0	DOR364
Brg5.2	BRGA	CIM	5	GC13	GC13–AN033D	16.7	3.6	15.9	32.6	–5.1	G19833
Brg5.1	BRGA	CIM	5	BMd028a	K124G–BMd028a	11.6	2.5	9.9	31.0	–4.9	G19833
Sbr1.1	SBRL	CIM	1	K123D	K123D–O20105G	13.2	2.9	9.9	39.8	106.6	DOR364
Sbr1.2	SBRL	IM	1	P1DB1D	P1DB1D–H1202D	13.1	2.84	20.3	21.4	118.2	DOR364
Sbr3.1	SBRL	CIM	3	I161G	pr599D–I161G	15.5	3.36	10.3	47.5	–79.5	G19833
Sbr5.1	SBRL	CIM	5	BMd028a	K124G–BMd028a	13.6	2.9	10.5	38.5	128.1	DOR364
Sbr6.1	SBRL	CIM	6	Bng094	Bng094–L0497D	12.9	2.81	9.3	48.8	–75.8	G19833
Sbr6.2	SBRL	CIM	6	AK061D	Bng046–G124G	15.4	3.3	14.2	40.3	168.9	DOR364
Sbr8.1	SBRL	CIM	8	DA59	AI1504G–DA59	14.4	3.1	11.7	40.3	114.0	DOR364
Sbr11.1	SBRL	IM / CIM	11	Bng187	V104D–W205G	17.6	3.83	13.4	49.3	–92.4	G19833
Rsb1.1	RSBRL	CIM	1	O20105G	K123D–O20105G	12.9	2.8	11.0	30.9	–3.8	G19833
Rsb2.1	RSBRL	CIM	2	V106D	V106D–O207G	12.2	2.65	15.7	39.7	–3.03	G19833
Rsb4.1	RSBRL	CIM	4	AN016D	BMd026–G1003D	17.6	3.8	14.0	37.5	–5.0	G19833
Rsb9.1	RSBRL	CIM	9	Bng065	BMd52–Bng065	12.6	2.7	18.3	51.7	–6.3	G19833
Rsb10.1	RSBRL	CIM	10	V10123D	GA26–V10123D	12.2	2.7	17.4	38.4	–5.6	G19833
Pup3.1	PAE	CIM	3	P076G	O204G–P076G	12.0	2.61	14.5	41.5	1.86	DOR364
Pup4.1	PAE	IM / CIM	4	P903G	Bng071–SW12.700	14.5	3.15	13.4	49.3	–1.10	G19833
Pup7.1	PAE	CIM	7	P9DB1D	P9DB1D–Bng052	9.6	2.08	12.2	47.1	–1.70	G19833
Pup9.1	PAE	CIM	9	V103G	V103G–U1002D	11.0	2.38	14.7	56.4	1.13	DOR364
Pup10.1	PAE	CIM	10	F602G	F602G–H201G	14.5	3.16	13.8	50.0	1.11	DOR364
Pup11.1	PAE	CIM	11	BMd022	BMd022–BMd33	10.1	2.19	9.3	49.4	–0.91	G19833

root gravitropic responses to phosphorus availability (Bonser *et al.* 1996; Liao *et al.* 2001). Our data also confirm that root gravitropism is associated with PAE. Under low P conditions in the field, shallow-rooted genotypes had greater shoot biomass and P content than deep-rooted genotypes (Tables 2, 3). Furthermore, several of the QTL controlling root gravitropic traits are linked to, or are pleiotropic with, those for PAE in the field (Fig. 3). These results indicate that genotypes with more gravitropic plasticity or shallower root systems can have superior PAE.

The focus of this report is the role of basal root shallowness in PAE. Although our results support the view that this trait is important for PAE, our mapping results also show that some QTL contributing to P efficiency in the field are not associated with basal root shallowness. This is not surprising given the fairly large number of distinct traits related to phosphorus acquisition, including traits for root morphology (notably root hairs), other architectural traits such as adventitious rooting and lateral branching, anatomical traits affecting root carbon economy (notably aerenchyma), rhizosphere modification through exudates, root phosphatase activity, microbial associations (notably arbuscular mycorrhiza), plant vigor, and no doubt other traits that have yet to be discovered (Trotta *et al.* 1991; Berta *et al.* 1995; Lynch and Beebe 1995; Borch *et al.* 1999; Fan *et al.* 2003; Miller *et al.* 2003; Vance *et al.* 2003; Lynch and Ho 2004). It is reasonable to assume that at least some of the QTL associated with PAE that are not associated with basal root shallowness may be associated with these other traits. The existence of multiple QTL associated with PAE through distinct mechanisms suggests that breeding strategies that combine several distinct traits of interest might be more successful than selection for shallow basal roots alone.

At least two factors contribute to the enhanced P efficiency of shallower root systems: (1) spatial coincidence of root foraging and resource distribution, since P availability is normally greatest in the topsoil and decreases with depth (Chu and Chang 1966; Pothuluri *et al.* 1986; Rubio *et al.* 2003) and (2) shallower root systems have lower intra-plant, inter-root competition (i.e. less overlap among neighboring root segments, Ge *et al.* 2000). This means that deep-rooted plants have a greater proportion of the root system that is not active in nutrient uptake. However, it has been observed that at agronomic planting densities, inter-root competition is more important in determining phosphorus uptake efficiency than inter-plant root competition (i.e. competition among neighboring plants; Rubio *et al.* 2001). Inter-root competition can reach values 50% higher than inter-plant root competition.

In addition to gravitropic responses to P availability, the plasticity of basal root gravitropism in response to P availability may also be related in other ways to PAE. In growth pouches, the BRGA of the shallow-rooted parent G19833 responded dynamically to phosphorus availability.

In the field, G19833 and shallow-rooted RILs responded dynamically to P availability, which permitted greater root exploitation of the topsoil, and greater biomass and P accumulation (Table 2). We hypothesise that plasticity is a positive trait, since it would permit the optimisation of architectural trade-offs in root function (Ho *et al.* 2004), such as between the acquisition of deep and shallow resources, depending on actual environmental constraints. Therefore, genes conferring root plasticity may be useful in plant breeding programs.

The utility of root shallowness may depend on several interacting factors in addition to P availability. For example, one disadvantage of shallow root systems could be a decreased ability for acquiring resources located deeper in the soil profile, such as water. Furthermore, since dry conditions near the soil surface are common in field conditions, mortality of fine roots in shallow root systems could be greater than in deeper ones (Espeleta and Eissenstat 1998). Root shallowness as discussed here focuses on the spatial distribution of basal roots. Other root types, such as tap roots, grow vertically regardless of nutrient availability. Different root types could play different roles in nutrient uptake. Functional complementation or compensation may occur among different root types. Water acquired from deep roots may leak into dry surface soil at night (Wraith and Baker 1991; Blum and Johnson 1992), allowing sustained nutrient uptake and root growth in the topsoil (Huang 1999).

The significant correlation of root distribution in growth pouches and field studies supports the validity of the growth pouch system for rapid screening of genotypes for root gravitropic traits (Table 4). In bean, basal roots form immediately near the base of the hypocotyl after the tap root elongates, and the tap root only grows vertically. Therefore, the spatial distribution of the seedling root system is largely determined by basal roots (Lynch and van Beem 1993). In previous work, we also observed a high correlation between growth pouches and 3-dimensional solid media such as sand and soil (Liao *et al.* 2001). Therefore, the growth pouch system is a valid approach for quantifying root gravitropic traits, especially at early growth stages. As seedlings age, it may also be necessary to consider adventitious roots as well (Miller *et al.* 2003). Bean plants form adventitious roots at about 10 d after planting, at which point basal roots are typically entering deeper soil. The significant differences in root gravitropism between the parents and $F_{5.7}$ progenies of the RILs indicate that these traits are heritable (Tables 1, 3). However, the inheritance of these traits was quantitative, with some degree of transgressive segregation (Fig. 2). Quantitative inheritance of root traits has been reported for other plant species, such as rice (Champoux *et al.* 1995; Price and Tomos 1997; Price *et al.* 1997; Hu *et al.* 2001; Wissuwa and Ae 2001; Zhang *et al.* 2001; Wissuwa *et al.* 2002). This may imply that the genes governing these traits are minor genes whose expression is easily subject to environmental influence, hence are difficult to detect with conventional methods (Stam 1998). With the molecular marker technology, we were able to detect QTL associated with the root gravitropic traits (Fig. 3). Our results show that there is normally more than one locus for each trait, each explaining less than 20% (R^2) of the total variability (Table 5). This confirms at the molecular level that genes conferring root gravitropic traits are quantitative in nature with minor effects. Transgressive segregation in the RIL population could be explained by the presence of both positive and negative alleles for root shallowness in both parents which was indeed the case for two phenotypic traits (BRGA and SBRL) as shown by QTL analysis (Table 5).

Three QTL for root gravitropic traits detected in growth pouches were associated with corresponding QTL for phosphorus uptake under low phosphorus conditions in the field, which supported the idea that root gravitropism contributed to phosphorus efficiency. It is interesting to note that all of the QTL for PAE in the field that were linked to QTL for root gravitropism traits come from G19833 (Table 5). This again suggests that root gravitropic traits were at least somewhat associated with PAE. On the other hand, many of the other QTL for the three root gravitropic traits measured in this study were independent of each other on the genetic map; and in many cases were unlinked to QTL for PAE in the field. One possible explanation for this may be that these traits are ontogenetically independent and are affected by different factors. For example, BRGA may not only be affected by phosphorus availability but also by other factors such as water availability, while SBRL and RSBRL may be affected by the size of the plant root system, basal root number and / or basal root extension, independent of a gravitropic response. Additionally, different basal roots at different positions along the tap root may differ in response to phosphorus availability. Indeed, the most basal pairs of basal roots have the greatest sensitivity to phosphorus availability (Zhang 2002). It is possible that a more detailed analysis of gravitropic traits may explain why some QTL for these traits are apparently not linked to PAE QTL while others are. We are able to conclude however that phosphorus acquisition is a complex process involving many traits to which gravitropic traits most likely make a contribution.

QTL for root gravitropism will be useful in the selection for P efficiency of common bean. Currently, the bean breeding program at the CIAT, which addresses the needs of resource poor farmers and consumers in Africa and Latin America, is using molecular markers for root traits derived from G19833 to select parental genotypes with phosphorus efficiency. Root gravitropic traits as measured in growth pouches may be useful selection criteria in this effort as well. Analogous processes may occur in roots of other crops, including other legumes (Bonser *et al.* 1996; Yang and Yan 1998, 1999) and rice (Kirk and van Du 1997). The technique and methodology

in the present study may also be applicable to the selection and breeding work for higher P efficiency in these and other crops.

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