# CROP ECOLOGY, MANAGEMENT & QUALITY

## Topsoil Foraging and Its Role in Plant Competitiveness for Phosphorus in Common Bean

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### ABSTRACT

**We evaluated the effect of root shallowness on interplant competition for phosphorus in common bean (*Phaseolus vulgaris* L.). Recombinant inbred lines (RILs) segregating for basal root gravitropism were evaluated in monogenetic and polygenetic stands with varying phosphorus availability in the field in South China and in solution culture and solid media in controlled environments. In the field, shallow-rooted RILs were more productive than deep-rooted RILs. Shoot biomass of these RILs almost doubled the deep-rooted ones when in competition. In the greenhouse, three treatments representing different soil phosphorus distributions were compared. Root shallowness did not confer any competitive advantage when phosphorus availability was uniformly low or uniformly high, but did confer a competitive advantage when phosphorus availability was concentrated in the topsoil. Shallow and deep-rooted RILs did not differ in response to phosphorus availability in solution culture where phosphorus is mixed and uniformly available. Our results demonstrate that basal root gravitropism, which is a specific root architectural trait under genetic control, is important for belowground competition in low phosphorus soils.**

Common bean is the most important food legume on earth, providing essential nutrients for hundreds of millions of people in developing countries (CIAT, 1987; Wortmann et al., 1998). Over half of global bean production occurs on severely phosphorus-deficient soils (Thung, 1990; Lynch and Beebe, 1995; Wortmann et al., 1998). Application of phosphate fertilizers is not an adequate solution to this problem because of rural poverty, poor access to appropriate fertilizers, and limited fertilizer efficacy in highly weathered soils. An alternative or complementary approach is the development of cultivars with superior growth and yield in soils with low phosphorus availability, or "phosphorus efficiency." Phosphorus efficient genotypes would yield better without fertilizers and would respond better to fertility inputs (Lynch, 1998). Significant genetic variation in phosphorus efficiency exists in bean (Lynch and Beebe, 1995; Beebe et al., 1997). Phosphorus efficiency in bean appears to be associated primarily with enhanced phosphorus acquisition from the soil through superior root growth and architecture rather than through microbial associations, chemical modification of the rhizosphere, or leaf acid phosphatase activity (Lynch and Beebe, 1995; Yan et al., 2001).

In most natural soils, phosphorus bioavailability is greater in surface or near-surface horizons than in the subsoil, because of deposition of phosphorus onto the soil surface in decayed leaves and other plant residues, as well as biological, chemical, and physical factors in the topsoil that favor phosphorus bioavailability. In agricultural soils, fertilization and cultivation increase phosphorus bioavailability in the topsoil, with only very slow movement of phosphorus into the subsoil in most cases. As a result, phosphorus availability usually declines substantially with soil depth (Chu and Chang, 1966; Keter and Ahn, 1986; Pothuluri et al., 1986). Root architectural traits that enhance the exploration and exploitation of surface horizons may therefore enhance phosphorus acquisition (Lynch and Brown, 2001).

Root gravitropism, one of the principal components of root architecture, may be an important mechanism responsible for phosphorus efficiency in bean. Root gravitropism is the tendency of a root to grow at a specific orientation with respect to gravity, or *Gravitropic Setpoint Angle* ("GSA," Firn and Digby, 1997). The GSA of the various classes of roots in a root system is an important determinant of root foraging at various depths in the soil profile. Root gravitropism may therefore be an important factor in topsoil foraging and therefore phosphorus acquisition in infertile soils (Lynch and Brown, 2001). The root system of common bean is composed of four root types: adventitious roots, basal roots, taproot, and lateral roots arising from the first three types (Fig. 1). The tap root has strong positive gravitropism and usually goes straight downwards. Adventitious roots arise from the hypocotyl and explore soil volumes close to the soil surface. Basal roots arise from the basal part of the root system. In conjunction with the lateral roots that emerge from them, basal roots usually comprise the majority of total root length. Basal root gravitropism is a key determinant of the overall shallowness of the root system, since basal roots form the scaffold on which most of the bean root system develops (Fig. 1) (Zobel, 1975). The growth of the basal roots with respect to gravity over time determines whether this part of the root system descends rapidly into the subsoil or remains in the topsoil (Lynch and Van Beem, 1993). Basal root gravitropism can be measured by the growth angle of the root axis or by the proportion of basal roots in the topsoil relative to the total amount of basal roots (Bonser et al., 1996, Liao et al., 2001).

Since basal root gravitropism is important for topsoil exploration, it is interesting that in bean and other le-

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gumes, genotypes inherently differ in basal root gravitropism, and phosphorus availability regulates basal root gravitropism in a genotype-dependent manner (Bonser et al., 1996). Genotypic differences in inherent growth angle and the responsiveness of growth angle to phosphorus availability were consistent in growth pouches in growth chambers, sand culture in greenhouses, and field studies, indicating that the effect was not overridden by mycorrhizal symbioses, soil resistance, or other environmental factors (Liao et al., 2001).

There are several lines of evidence suggesting that this trait enhances topsoil foraging and thereby phosphorus acquisition efficiency. The geometric simulation model SimRoot was used to model the effect of changing basal root gravitropism on interroot competition for phosphorus (Ge et al., 2000). This study showed that in soils with uniform phosphorus distribution, shallower root systems explored more soil per unit of root biomass than deeper systems, because shallower systems have more dispersed basal roots and therefore less interroot competition, which occurs when neighboring roots have overlapping phosphorus depletion zones (Ge et al., 2000). In stratified soils with more phosphorus in the topsoil, the simulations showed that shallower root systems acquired more phosphorus than deep ones, by concentrating root foraging in the topsoil (Ge et al., 2000). These modeling results are supported by the significant correlation of basal root growth angle in young bean genotypes in growth pouches with their yield in field trials in low phosphorus tropical soils (Bonser et al., 1996). In comparisons of individual plants grown in pots of soil, genotypes with shallower basal roots had greater phosphorus uptake than those with deeper root systems (Liao et al., 2001).

The impact of basal root gravitropism on plant performance in the field is more complex, because of tradeoffs between topsoil resources such as phosphorus with deeper resources such as water, as well as competitive interactions with neighboring plants. In the case of diffusion-mobile nutrients, uptake at the root surface creates a zone of depleted soil in the rhizosphere. Belowground competition among roots for diffusion-mobile nutrients occurs when their individual depletion volumes overlap, causing a reduction in nutrient uptake (Robinson, 1991). It is therefore possible that a bean genotype with shallow basal roots would have superior topsoil foraging and thus phosphorus acquisition in isolation, but in monogenetic stands would suffer increased competition from neighboring plants. Geometric modeling indicates that basal root gravitropism may have a substantial effect on interplant competition for phosphorus in common bean (Rubio et al., 2001). In this study, we focus on the effect of root shallowness on interplant competition for phosphorus to test the following predictions: (i) in monogenetic stands, shallow rooted genotypes will be more efficient than deeper ones in phosphorus-limited soils and (ii) in polygenetic mixtures of shallow and deep rooted genotypes, shallow rooted genotypes will have a competitive advantage.

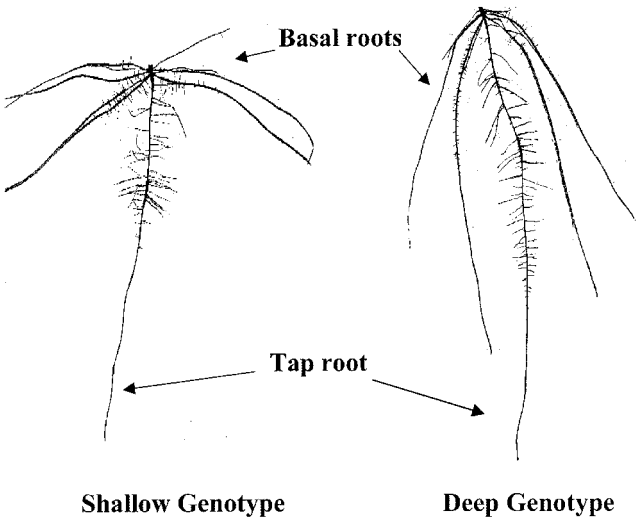


**Fig. 1. Two bean root systems contrasting in basal root gravitropism 6 d after germination.**

## MATERIALS AND METHODS

### Plant Material

In previous studies, we found large genetic variability for root gravitropism in common bean cultivars (Bonser et al., 1996; Liao et al., 2001). Two genotypes contrasting in root gravitropism (G19833, shallow; DOR364, deep) but similar in growth rate under field conditions (Rubio et al., 2001) were chosen as parents in this study. DOR364 pertains to race *M* of the Mesoamerican gene pool, and G19833 is of race Nueva Granada of the Andean gene pool (Singh et al., 1991). DOR364 is a deep-rooted, high yielding cultivar, while G19833 is also a high yielding landrace with exceptional phosphorus efficiency, having a shallow root system (CIAT, 1990; Lynch and Beebe, 1995; Yan et al., 1995; Bonser et al., 1996; Liao et al., 2001; Rubio et al., 2001). Progenies from the cross between G19833 and DOR364 were advanced by single seed descent to the $F_{5.10}$ generation as recombinant inbred lines (RILs)(Liao et al., 1999). RILs are advantageous for comparisons of traits controlled by an unknown number of genes, because each RIL represents a particular combination of genes from a common pair of parents. RILS are a standard genetic tool used in generating genetic maps of QTLs, and many papers have been published describing them and their use (Groh et al., 1998; Arahana et al., 2001). Isolines are not useful for analysis of quantitative traits, since they differ in one gene, and quantitative traits by definition are governed by many genes. Eighty-seven RILs were obtained in this way and then grown in growth pouches and sand culture to observe the basal root growth angle and the proportion of basal roots in the surface layers, parameters of root gravitropism (Liao et al., 1999). From this screening, four RILs with contrasting root architecture but similar growth rate were selected for the present study. The two shallow-rooted selections were RILs 17 and 7 and the two deep-rooted selections were RILs 24 and 38.

### Field Experiment

We evaluated the association of root architecture and plant performance in monogenetic and polygenetic stands, in a field study in Guangzhou, China (26°06′N, 113°15′E), in June and July 1999. The soil was a highly weathered sandy loam (Typic Paleudult). The pH was 4.6, which was adjusted to 5.8 2 wk before the experiment by adding calcitic lime (1500 kg $ha^{-1}$).

**Table 1. *F* values and significance levels for the ANOVAs in the field experiment at Guangzhou with common bean genotypes. Factors were phosphorus (medium and high), genotype (RILs 17 and 24, and 7 and 38, in two separated experiments), and competition (monoculture and competition). In the competition treatments, root parameters (i.e., number of root intersections, and number and proportion of root intersections in the top 5 cm) of competing plants were analyzed as a group because they could not be separated. In this case, the competition treatment was included as the third level of the genotype factor.**

| | Source | | | | | |
|---|---|---|---|---|---|---|
| | Phosphorus | Genotype | Competition | P×G | P×C | G×C |
| | RILs 17 (shallow) and 24 (deep) | | | | | |
| Shoot biomass 20 DAP | 1.18 | 9.09** | 1.69 | 4.59* | 1.32 | 0.13 |
| Shoot biomass 35 DAP | 9.94*** | 51.11*** | 2.1 | 6.31* | 0.52 | 10.71*** |
| Plant height | 1.17 | 15.43*** | 0.12 | 5.18* | 0.40 | 0.00 |
| Shoot P concentration | 7.29* | 0.54 | 0.04 | 0.00 | 0.00 | 0.07 |
| Number of basal roots | 1.45 | 0.37 | 0.18 | 0.00 | 0.02 | 1.40 |
| Number of adventitious roots | 0.26 | 2.98 | 0.06 | 1.96 | 0.27 | 0.06 |
| Number of root intersections | 0.09 | 0.84 | | 0.49 | | |
| Number of root intersections in the top 5 cm | 1.54 | 5.74** | | 1.40 | | |
| Proportion of roots in the top 5 cm | 0.82 | 4.16* | | 0.56 | | |
| | RILs 7 (shallow) and 38 (deep) | | | | | |
| Shoot biomass 20 DAP | 9.24** | 4.46 | 0.81 | 6.06* | 1.74 | 1.82 |
| Shoot biomass 35 DAP | 1.25 | 5.2* | 0.19 | 0.02 | 0.02 | 5.03* |
| Plant height | 0.98 | 0.77 | 3.38 | 0.77 | 0.18 | 0.06 |
| Shoot P concentration | 5.94* | 0.35 | 0.07 | 0.15 | 1.77 | 0.31 |
| Number of basal roots | 0.24 | 8.00** | 0.35 | 0.20 | 2.19 | 2.27 |
| Number of adventitious roots | 0.10 | 1.77 | 0.86 | 0.93 | 4.67 | 0.05 |
| Number of root intersections | 2.10 | 1.46 | | 1.11 | | |
| Number of root intersections in the top 5 cm | 2.80 | 7.36** | | 0.09 | | |
| Proportion of roots in the top 5 cm | 7.26* | 4.79* | | 0.90 | | |

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

All plots received a preplant broadcast fertilizer application of 180 kg N $ha^{-1}$ as urea and 200 kg K $ha^{-1}$ as KCl. No irrigation was required since rains occurred almost daily. Seeds were planted in rows 1 m apart and the distance between plants in the rows was 5 cm. No shading between plants of different rows was observed during the experiment.

Two pairs of contrasting RILs were evaluated. Each pair constituted an independent experiment composed of one shallow- and one deep-rooted genotype. Random assignment of the RILs resulted in pairings of 17 vs. 24 and 7 vs. 38. Each experiment included three factors: competition, genotype, and phosphorus availability. The competition factor had two treatments: monoculture (all plants in the row belonging to the same genotype) and competition (plants of different geno-

**Table 2. Average values and standard errors (between brackets) for several above and belowground parameters in the field experiment at Guangzhou. Factors are phosphorus (medium and high), genotype (RILs 17 and 24, and 7 and 38, in two separated experiments), and competition (monoculture and competition). In the competition treatments, root parameters (i.e., number of root intersections, and number and proportion of root intersections in the top 5 cm) of competing plants were analyzed as a group because they could not be separated.**

| | Medium phosphorus | | | | High phosphorus | | | |
|---|---|---|---|---|---|---|---|---|
| | Monoculture | | Competition | | Monoculture | | Competition | |
| RILs 17 (shallow) and 24 (deep) | Shallow | Deep | Shallow | Deep | Shallow | Deep | Shallow | Deep |
| Shoot biomass 20 DAP (g) | 1.13 (0.09) | 0.79 (0.2) | 0.89 (0.14) | 1.03 (0.23) | 1.30 (0.21) | 1.05 (0.2) | 1.35 (0.14) | 0.64 (0.27) |
| Plant height (cm) | 35.6 (1.17) | 32.91 (2.12) | 36.80 (2.10) | 34.60 (2.50) | 37.37 (1.71) | 29.11 (3.61) | 37.25 (1.70) | 27.72 (3.50) |
| Shoot P concentration (%) | 0.28 (0.04) | 0.27 (0.04) | 0.29 (0.01) | 0.27 (0.03) | 0.34 (0.03) | 0.32 (0.02) | 0.33 (0.02) | 0.34 (0.03) |
| Number of basal roots | 7.40 (0.89) | 6.80 (0.57) | 6.50 (0.64) | 7.20 (0.65) | 7.80 (0.99) | 7.60 (0.54) | 7.25 (1.18) | 8.00 (1.08) |
| Number of adventitious roots | 16.70 (3.01) | 15.00 (3.35) | 14.33 (2.57) | 15.20 (1.62) | 16.00 (3.95) | 20.10 (7.35) | 10.00 (2.04) | 9.25 (1.44) |
| | | | Shallow + deep | | | | Shallow + deep | |
| Number of root intersections | 73.00 (6.00) | 66.4 (3.95) | 73.00 (10.45) | | 79.20 (5.51) | 64.60 (15.23) | 61.25 (6.22) | |
| Number of root intersections in the top 5 cm | 17.62 (2.25) | 10.60 (3.05) | 18.2 (3.45) | | 19.2 (4.31) | 8.25 (2.95) | 10.00 (1.45) | |
| | Medium phosphorus | | | | High phosphorus | | | |
| | Monoculture | | Competition | | Monoculture | | Competition | |
| RILs 7 (shallow) and 38 (deep) | Shallow | Deep | Shallow | Deep | Shallow | Deep | Shallow | Deep |
| Shoot biomass 20 DAP (g) | 0.96 (0.09) | 0.68 (0.27) | 1.25 (0.02) | 0.57 (0.08) | 1.15 (0.24) | 0.66 (.08) | 1.05 (0.12) | 1.46 (0.15) |
| Plant height (cm) | 32.10 (2.66) | 31.25 (3.11) | 30.60 (0.76) | 27.60 (2.36) | 34.20 (2.05) | 29.30 (0.20) | 31.50 (2.47) | 32.00 (1.41) |
| Shoot P concentration (%) | 0.28 (0.04) | 0.28 (0.08) | 0.31 (0.02) | 0.29 (0.01) | 0.35 (0.01) | 0.34 (0.01) | 0.33 (0.01) | 0.31 (0.03) |
| Number of basal roots | 9.75 (1.20) | 7.4 (0.48) | 9.20 (0.96) | 6.80 (0.65) | 9.50 (0.88) | 5.33 (0.95) | 8.00 (0.71) | 10.00 (3.53) |
| Number of adventitious roots | 11.75 (3.58) | 14.00 (4.31) | 16.40 (3.03) | 18.00 (4.56) | 20.50 (2.52) | 11.00 (2.82) | 18.50 (4.60) | 13.00 (1.41) |
| | | | Shallow + deep | | | | Shallow + deep | |
| Number of root intersections | 55.0 (9.85) | 35.50 (7.40) | 57.22 (9.42) | | 61.2 (6.63) | 57.00 (12.01) | 56.50 (1.06) | |
| Number of root intersections in the top 5 cm | 20.00 (3.72) | 10.50 (2.17) | 15.00 (4.12) | | 16.80 (3.34) | 6.33 (2.49) | 11.00 (3.53) | |

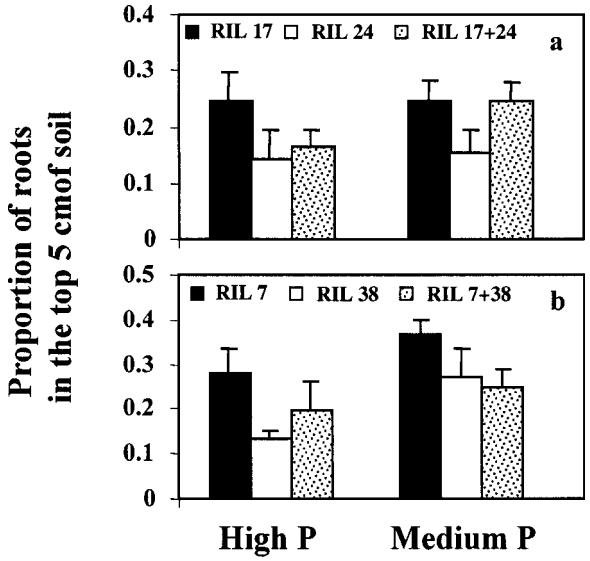


**Fig. 2. Ratio of root intersections of common bean plants in the top 5 cm of the soil to the total number of root intersections as affected by genotype, competition and phosphorus availability in the field in China. Two pairs of RILs, 17 and 24 (a) and 7 and 38 (b), are compared. Each bar represents the mean of five replicates; error bar represents the standard error of the mean. In the competition treatments roots of competing plants were analyzed as a group because they could not be separated.**

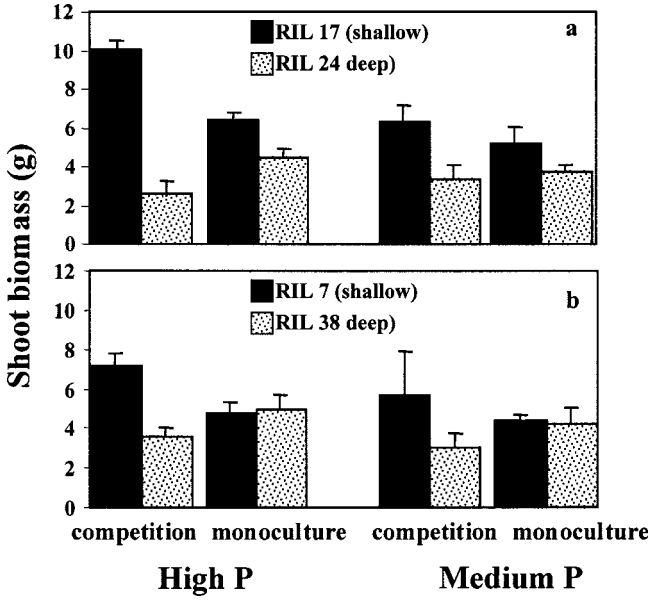


**Fig. 3. Shoot biomass of common bean as affected by genotype, competition and phosphorus availability in the field at Guangzhou, China. Shallow rooted genotypes (RIL 17 in panel A, RIL 7 in panel B), and deep rooted genotypes (RIL 24 in panel A, RIL 38 in panel B) were included. Each bar represents the mean of five replicates; error bar represents the standard error of the mean.**

types intercalated in the row). Genotypes studied were RILs 17 and 24 in one experiment and 7 and 38 in the other. The phosphorus levels were control (medium P, no phosphorus added) and fertilized (high P, 160 kg P $ha^{-1}$ added as triple superphosphate). Both phosphorus levels had a marked increase in concentration of phosphorus toward the topsoil. The control treatment plots had 6.7, 2.4 and 1.2 mg P $kg^{-1}$ soil (Bray II) in the 0- to 10-, 10- to 20-, and 20- to 30-cm soil layers, respectively. The fertilized soil had 35.9, 2.3, and 1.2 mg P $kg^{-1}$ soil (Bray II) in the 0- to 10-, 10- to 20-, and 20- to 30-cm soil layers, respectively. Applied phosphorus fertilizer was not enough to obtain maximum yield, although it was sufficient to significantly increase yield. There were a total of five replications, with each replication constituting a block in a randomized complete block design. Each experimental unit had 30 plants (length of the plot 1.5 m, plants 5 cm apart). Plants were harvested at 20 and 35 d after planting. Plants were not evaluated during reproduction to avoid possible confounding effects of differential flowering dates. At each harvest, nine plants were harvested, and four border plants were discarded. Plant biomass, number of leaves, plant height, and number of basal and adventitious roots were measured. Shoot dry weights were taken after 3 d of drying at 65°C. Tissue phosphorus content was measured spectrophotometrically (Murphy and Riley, 1963) on dry-ashed tissue. To evaluate the competitive ability, the competitive index was calculated as follows:

Competitive index ($A$) = (Shoot biomass $A$ in competition/Shoot biomass $B$ in competition)/(Shoot biomass $A$ in monoculture/Shoot biomass $B$ in monoculture)

Where $A$ and $B$ are the compared genotypes. Values close to 1 signify no competitive advantage between genotypes. Values of the index greater than 1 signify that Genotype $A$ is favored by competition, whereas lower values signify that Genotype $A$ suffers a competitive disadvantage.

Before the final harvest, soil trenches were made to analyze the spatial distribution of the roots. A modified version of the profile wall method described by Schuurman and Goedewaagen (1971) was used. The width of the trenches was 46 cm and the depth was determined according to the maximum depth of the basal roots. The trenches were excavated parallel to the plant row, 0.10 m apart from it. The walls were gently abraded to reveal the roots. Acetate sheets were suspended on the soil wall, and the roots were traced on the sheets with an indelible marker. The number of total root intersections, and the number of intersections in the top 5 cm were measured. The proportion of basal and adventitious roots in the top layer of the soil profile relative to the total amount of roots has been a useful measure of root gravitropism (Liao et al., 2001; Rubio et al., 2001)

**Table 3. Competitive index for shallow and deep-rooted RILs of common bean in the field at Guangzhou and in solid media under controlled conditions. Factors were (i) field experiment: phosphorus (medium and high), genotype (RILs 17 and 24, and 7 and 38, in two separated experiments), and competition (monoculture and competition); (ii) Solid media experiment: phosphorus (low, high, and stratified phosphorus), genotype (RILs 7 and 38), and competition (monoculture and competition).**

| | Phosphorus treatments | | |
|---|---|---|---|
| **Field experiment** | | **Medium** | **High** |
| **RIL 17 (shallow)** | | | |
| **RIL 17** | | **1.35** | **2.68** |
| **RIL 24** | | **0.73** | **0.37** |
| **RIL 7 (shallow)** | | | |
| **RIL 7** | | **1.77** | **2.09** |
| **RIL 38** | | **0.56** | **0.48** |
| **Solid media** | **Low phosphorus** | **High phosphorus** | **Stratified** |
| **RIL 7** | **0.76** | **0.98** | **1.12** |
| **RIL 38** | **1.30** | **1.01** | **0.88** |

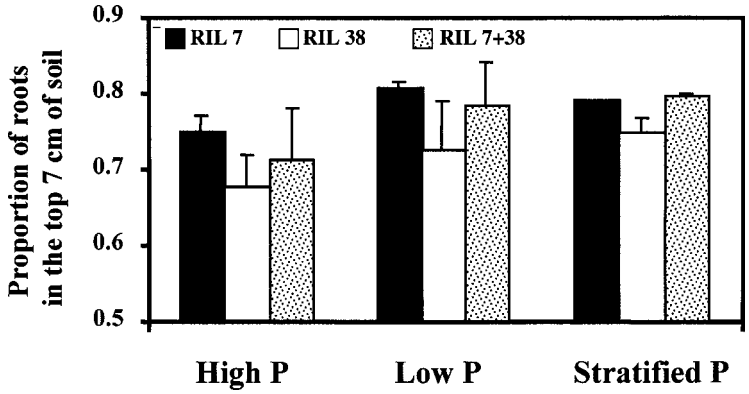


**Fig. 4. Ratio of root intersections of common bean plants in the top 7 cm of the soil to the total number of root intersections as affected by genotype, competition, and phosphorus availability in solid media in the greenhouse. Each bar represents the mean of three replicates; error bar represents the standard error of the mean. In the competition treatments roots of competing plants were analyzed as a group because they could not be separated.**

## Solid Media Experiment

An experiment was conducted in greenhouse conditions, in which the localization and availability of phosphorus were explicitly controlled, and phosphorus stress could be applied in the absence of other stresses that commonly occur in the field. The experimental system consisted of nine plastic 18-L (60 cm deep) containers filled with equal volumes of sand, perlite, and vermiculite. The media was mixed with solid-phase-buffered phosphorus (Lynch et al., 1990) to provide three different regimes of buffered phosphorus availability: low phosphorus (0.2 $\mu M$ phosphorus, uniform in all the growing media); high phosphorus (50 $\mu M$ phosphorus, uniform in all the growing media) and stratified phosphorus (50 $\mu M$ phosphorus in the top 7 cm and 0.2 $\mu M$ phosphorus below 7 cm). In all treatments, plastic grids (with 2-mm square holes) were installed at 7 cm from the top to separate the growing media in two layers, topsoil and subsoil. This system was located in a temperature controlled (approximately 30°C daytime and approximately 25°C nighttime) greenhouse at University Park, PA, USA (40°85N; 77°83W). Plants were automatically irrigated once a day with a nutrient solution composed of (in $\mu M$) 1500 $KNO_3$, 1200 $Ca(NO_3)_2$, 400 $NH_4NO_3$, 25 $MgCl_2$, 5 Fe-Na-EDTA, 500 $MgSO_4$, 300 $K_2SO_4$, 300 $(NH_4)_2SO_4$, 1.5 $MnSO_4$, 1.5 $ZnSO_4$, 0.5 $CuSO_4$, 0.143 $(NH_4)_6Mo_7O_{24}$, and 0.5 $Na_2B_4O_7$. To minimize accumulation of nutrients in the growing media, the irrigation system was set up to add sufficient solution to displace the solution present in the media before the irrigation event. Treatments were arranged in a randomized block design with three factors and three replications. The factors were phosphorus (three levels), genotype (two levels), and competition (two levels). Genotypes analyzed were RILs 7 and 38 and the competition levels were monoculture and competition. Seeds of the selected RILs were sterilized, germinated in an incubator at 25°C, and then the seedlings were transferred to the solid culture system. Seedlings were planted 5 cm apart, in a single row at the center of the container. In the competition treatment, plants of both genotypes were intermixed.

At 28 d after transplanting, plant shoots were harvested and dry weights were taken after 3 d at 60°C. Meanwhile, the roots from each layer (top 7 cm and below 7 cm) were rinsed from the soil, dried, and weighed.

## Solution Culture Experiment

To evaluate if inherent differences in growth rate exist among the RILs, we performed an experiment in solution culture, in which root architecture has no specific effects on nutrient uptake since nutrients are evenly distributed in the growing media and roots have no pattern of spatial distribution. Seeds of RILs 7 and 38 were sterilized, germinated in an incubator at 25°C, and then transferred to a nutrient solution. The system was composed of eight plastic containers (0.5 by 0.4 by 0.25 m) which were covered by a lid with 12 evenly spaced holes. Twelve plants (six of each genotype) were transferred to each container. Genotypes 7 and 38 were intermixed; hence plants of one genotype were surrounded by plants of the other genotype. Thus, each phosphorus treatment was replicated four times, with six subsamples of each genotype within each phosphorus treatment. The system was placed in the same greenhouse and under the same conditions as described above. The container was filled with a solution containing the same nutrient concentration of the solution used for the solid media experiment. Solution pH was maintained between 5.8 and 6.5 by additions of KOH or HCl. The nutrient

**Table 4. *F* values and significance levels for the ANOVAs of the solid media experiment with common bean genotypes. Factors were phosphorus (low, high, and stratified P), genotype (RILs 7 and 38), and competition (monoculture and competition). In the competition treatments, roots of competing plants were analyzed as a group because they could not be separated. In this case, the competition treatment was included as the third level of the genotype factor.**

| | Source | | | | | |
|---|---|---|---|---|---|---|
| | Phosphorus | Genotype | Competition | P×G | P×C | G×C |
| Leaf area | 25.28*** | 7.90** | 3.41 | 1.25 | 0.33 | 0.03 |
| Shoot biomass | 29.38*** | 22.72*** | 1.63 | 0.50 | 0.71 | 0.66 |
| Root biomass | 0.66 | 10.32 | | 1.87 | | |
| Total biomass | 8.61*** | 6.72** | | 1.32 | | |
| Root/shoot ratio | 2.63 | 2.90 | | 0.91 | | |
| Proportion of roots in the top 5 cm | 0.88 | 4.18* | | 1.02 | | |

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

solution was renewed every 5 d. Two phosphorus treatments were included: low (0.1 $\mu M$ phosphorus) and high (70 $\mu M$ phosphorus). These concentrations were obtained by the addition of $KH_2PO_4$.

At 28 d after transplanting, plant shoots and roots were harvested and dry weights were taken after 3 d at 60°C. Roots were stained with 0.16% (v/v) neutral red dye before being scanned. Root length was quantified with a computer image analysis program DT-Scan (Delta-T Devices Inc. Ltd., England).

### Measurement of Root Hairs

Seeds of the two genotypes were surface sterilized for 1 min in 10% (v/v) NaOCl before germination. Seeds were germinated in germination paper and soaked in 0.5 m$M$ CaSO4 in darkness at 25°C. Seven days later, uniform seedlings were transplanted into 100-L hydroponic tanks with nutrient solution, which was composed of (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 $\mu M$) 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 parental materials were exposed to three phosphorus levels, which were 0.2, 2, and 1000 $\mu M$ phosphorus as $KH_2PO_4$, while the RILs were treated with one low-P level (0.2 $\mu M$ phosphorus). For the low phosphorus (0.2 and 2 $\mu M$) treatments, a solid phase buffer (phosphorus adsorbed to activated aluminum oxide) was used to supply realistically low yet stable concentrations of phosphate (Lynch et al., 1990). The solution was well aerated and the pH was maintained between 5.8 and 6.0 with daily additions of KOH or HCl. Plants were grown in a greenhouse at Penn State with an average temperature of 29/20°C (day/night), relative humidity 48/83% (day/night) and average photosynthetically active radiation between 500 and 1000 μmol photons $m^{-2}$ $s^{-1}$ during the day.

Plants were harvested on the 14th day after transplanting and the roots were preserved in 25% ethanol immediately after harvest. Ten first-order lateral roots were randomly selected from both basal and tap roots of each plant, and three plants were sampled for each treatment. The roots were stained and observed with a stereomicroscope (Nikon SMZ-U, Japan) equipped with a CCD camera. A section of the root with representative root hair density and length was selected for image capture. The images were imported to Metamorph imaging software (Universal Imaging Company, West Chester, PA) and DT-Scan software (Delta-T Devices Ltd., England) for quantitative analysis of the root hairs. Root hair density (number $mm^{-1}$ root), average root hair length (mm), and total root hair length (m/plant) were quantified.

### Data Analysis

Because of the difficulty of isolating individual roots in treatments involving two different genotypes, the measurements of root parameters in the competition treatments included no individual data for each genotype. Instead, reported data correspond to roots of both genotypes. Consequently, the factors in the ANOVAs were different for the shoot parameters and for root parameters. In the case of the root parameters, the competition factor was eliminated. The collected data for the competition treatments were included as a third level in the genotype factor. Data from the three experiments were analyzed as a randomized block design.

## RESULTS

### Field Experiment

#### Comparison between RILs 17 and 24

No basal roots were found below a 0.5-m depth either in this or in the 7 vs. 38 comparison. The maximum depth

**Table 5. Average values and standard errors for several above and belowground parameters in the solid media experiment. Factors were phosphorus (low, high, and stratified phosphorus), genotype (RILs 7 and 38), and competition (monoculture and competition). In the competition treatments, roots of competing plants were analyzed as a group because they could not be separated.**

| | Low phosphorus | | | | High phosphorus | | | | Stratified phosphorus | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Monoculture | | Competition | | Monoculture | | Competition | | Monoculture | | Competition | |
| | RIL 7 | RIL 38 | RIL 7 | RIL 38 | RIL 7 | RIL 38 | RIL 7 | RIL 38 | RIL 7 | RIL 38 | RIL 7 | RIL 38 |
| Leaf area ($cm^2$) | 130 (13.6) | 125 (39.1) | 116 (16.5) | 109 (8.4) | 223 (20.7) | 192 (25.2) | 236 (58.8) | 178 (2.3) | 169 (16.8) | 121 (24.0) | 145 (24.1) | 92 (7.2) |
| | | | 7 + 38 | | | | 7 + 38 | | | | 7 + 38 | |
| Root biomass (g) | 0.26 (0.09) | 0.14 (0.04) | 0.30 (0.10) | | 0.34 (0.11) | 0.22 (0.07) | 0.22 (0.09) | | 0.35 (0.16) | 0.19 (0.08) | 0.27 (0.07) | |
| Total biomass (g) | 0.86 (0.16) | 0.56 (0.09) | 0.91 (0.09) | | 1.32 (0.15) | 1.07 (0.12) | 1.03 (0.14) | | 1.21 (0.32) | 0.78 (0.21) | 1.11 (0.11) | |
| Root/shoot | 0.43 (0.12) | 0.30 (0.15) | 0.56 (0.18) | | 0.36 (0.11) | 0.26 (0.12) | 0.29 (.11) | | 0.37 (0.11) | 0.29 (0.06) | 0.36 (0.11) | |
| Roots in the top 5 cm (g) | 0.21 (0.07) | 0.10 (0.04) | 0.25 (0.10) | | 0.26 (0.09) | 0.16 (0.06) | 0.15 (0.05) | | 0.28 (0.13) | 0.14 (0.06) | 0.22 (0.06) | |

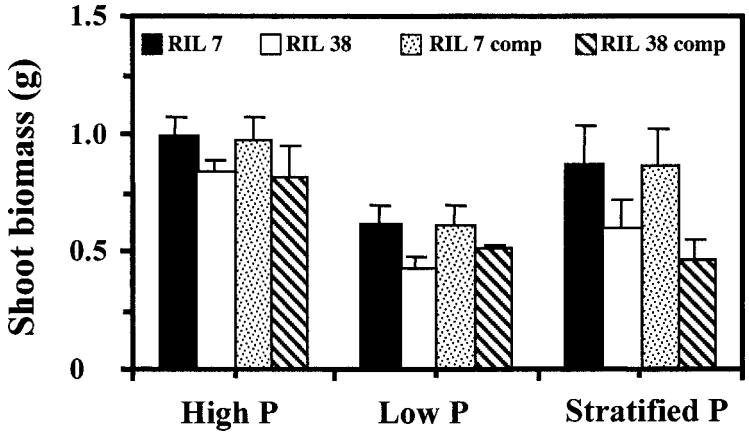


**Fig. 5. Shoot biomass of common bean as affected by genotype, competition and phosphorus availability in solid media in the greenhouse. Each bar represents the mean of three replicates; error bar represents the standard error of the mean.**

of the root systems, attained by the taproot system, varied from 1 to 2 m. These genotypes did not differ in the total number of root intersections although they showed significant differences in the vertical distribution of the root system (Table 1, 2). RIL 17 had a shallower root system, as reflected by the number of root intersections in the first 5 cm of the soil and the proportion of these intersections over the total number of root intersections (Table 1, Fig. 2). This difference in root gravitropism was not affected by the phosphorus availability: no influence of the phosphorus level was detected between genotypes in any of the measured root parameters (Table 1). Values for the proportion of roots in the top 5 cm of the soil when both genotypes were grown in competition were in between the values found for both genotypes grown in monoculture. No treatment effects were observed on the number of basal and adventitious roots (Table 1).

Shoot biomass production and P concentration after 35 d of growth was enhanced by phosphorus addition (Table 1). In both competition and monoculture stands, shoots of RIL 17 had a greater accumulation of biomass than shoots of RIL 24, the difference between genotypes being larger in the competition treatments (Fig. 3). Plant height values varied from 27.7 to 37.4 cm. Maximum differences in this parameter between genotypes were detected in the high P-competition treatment (Table 2). The competitive index indicated that, at both phosphorus levels, the shallower genotype (17) was greatly favored by competition (Table 3).

**Table 6. *F* values and significance levels (between brackets) for the ANOVAs of the hydroponics experiment with common bean genotypes. Factors were phosphorus (low and high) and genotype (RILs 7 and 38).**

| | Phosphorus | Genotype | P×G |
|---|---|---|---|
| Leaf area | 186.44*** | 0.15 | 0.38 |
| Shoot biomass | 134.22*** | 1.32 | 0.80 |
| Root biomass | 62.03*** | 6.07* | 3.21 |
| Total biomass | 128.73*** | 2.22 | 1.28 |
| Root length | 1.91 | 69.60*** | 0.36 |
| Root/shoot | 36.89*** | 3.78 | 0.06 |
| Specific root length | 19.83*** | 15.90*** | 5.25* |

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

### Comparison between RILs 7 and 38

Neither the phosphorus nor the genotype factors affected the total number of root intersections in this pair of genotypes (Table 1). RIL 7 had a shallower root system than RIL 38, with more than 30% of the roots localized in the first 5 cm of the soil profile (Fig. 2). Phosphorus fertilization modified the pattern of root architecture; in fertilized soil roots were deeper (Table 1). No clear treatment effects on the number of basal roots and adventitious roots were detected (Table 2). Phosphorus addition increased the plant phosphorus concentration and the shoot biomass production in 20-d-old plants (Tables 1, 2). In 35-d-old plants, genotype effects on shoot biomass depended on whether plants were grown in competition or monoculture stands. When grown in monoculture, no significant differences in shoot biomass accumulation among RILs were found (Fig. 3). In competition and at both phosphorus levels, 7 had a greater accumulation of shoot biomass than 38. The competitive advantage of the shallower genotype was clearly expressed by the competitive index, which indicated that the RIL 7 was favored by competition (Table 3). Plant height did not explain this competitive advantage, since RILs did not show any significant difference on this parameter (Table 2).

## Solid Media Experiment

Compared with the field experiment, plants growing in this experiment had a greater proportion of roots in

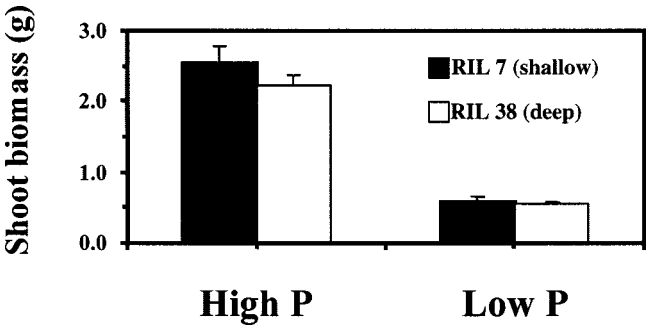


**Fig. 6. Shoot biomass of common bean as affected by genotype and phosphorus availability in solution culture. Each bar represents the mean of four replicates; error bar represents the standard error of the mean.**

the upper layers (Fig. 4). More than 60% of the roots were present in the top 7 cm of the soil. Nevertheless, both genotypes still differed in root shallowness. This difference was consistent with what we had observed in the field since RIL 7 had a shallower root system than 38. In the competition treatments, the distribution of roots tended to be in between values found in monoculture (Fig. 4). There were no effects of the phosphorus level on the proportion of roots in the topsoil (Table 1). Total root biomass was quite variable, although a tendency for RIL 7 to accumulate more root biomass than RIL 38 was detected (Table 4, 5).

Phosphorus supply strongly enhanced the leaf area and shoot biomass accumulation of the studied genotypes (Tables 4, 5, Fig. 5). In the layer phosphorus treatment, shoot biomass was intermediate between the high phosphorus and the low phosphorus treatments. The two studied RILs showed different responses to phosphorus and competition. At all phosphorus levels, RIL 7 had a greater biomass accumulation than RIL 24. The biggest differences between genotypes were found in the layer phosphorus treatment, in both monoculture and competition treatments (Table 1, Fig. 5). Only layer phosphorus plants showed differences between RILs in the leaf area, which was much larger in the RIL 7 than in 38 (Tables 4,5). The competitive index indicated that competitiveness was strongly influenced by the phosphorus level (Table 3). While under high phosphorus conditions all of the RILs were equally competitive, under low phosphorus conditions RIL 38 was a stronger competitor than RIL 7 and the opposite occurred in the layer phosphorus treatment (Table 3).

### Solution Culture Experiment

Plants of this experiment were much larger than plants of the same age grown in the solid media experiments. This resulted in a more intense shading among neighboring plants. However, almost no differences in plant growth between RILs was detected in solution culture (Table 6). Data from Table 2 and Fig. 6 show that no significant differences between genotypes were detected in either shoot biomass or leaf area under either phosphorus level. Growth of both RIL 7 and RIL 38 was strongly promoted by phosphorus supply and this effect was much larger in shoots than in roots (Tables 6, 7). Consequently, high phosphorus plants showed a lower root/shoot ratio (Table 7). Phosphorus deficiency also increased the specific root length, a parameter that showed higher values in RIL 7 than in 38 (Table 6). Although RIL 38 had a higher root hair density than RIL 7 (117.4 $mm^{-1}$, SE = 24.4 and 58.4 $mm^{-1}$, SE = 29.6, respectively), there were no differences between genotypes in the total root hair length (9.95 m, SE = 4.63 for RIL 7 and 18.1 m, SE = 7.40 in RIL 38).

## DISCUSSION

The proportion of roots in the top 5 cm of the soil of RILs 17 and 7 almost doubled the proportion found in their counterparts, RILs 24 and 38. Field results were consistent with results from solid media in the greenhouse, indicating that this architectural trait is a robust phenotype. No relationship was found between root shallowness and the number of adventitious or basal roots. Moreover, the deep-rooted RIL 38 had a higher number of basal roots than RIL 7. These results are consistent with earlier studies, which concluded that root shallowness is determined by the root trajectories defined by the angles of the basal roots, rather than by the number of basal roots (Bonser et al., 1996; Liao et al., 2001). Phosphorus deficiency significantly increased the root/shoot ratio in the solution culture experiment. It is known that phosphorus regulates biomass allocation patterns as it was shown for beans by Cakmak et al. (1994) and Nielsen et al. (2001). Phosphorus stress decreased root angles (i.e., created a shallower root system) in the 7 vs. 38 field comparison. However, the phosphorus effect on root gravitropism was not observed in the other comparisons performed in the present work. We did not detect a proliferation of roots in the layer where phosphorus was concentrated, a common response in other species (Caldwell, 1994). This was clearly confirmed in the solid media experiment, in which plants in the stratified phosphorus treatment did not show any clear pattern of root proliferation in phosphorus-rich horizons. Solution culture eliminates many of the effects of root architecture on nutrient uptake, since nutrients are homogeneously distributed in the nutrient solution and no pattern of root spatial distribution exists. However, it does not eliminate the effects of other root parameters related to nutrient efficiency (Chapin and Van Cleeve, 1989), such as the root hairs or the specific root length. We observed that the deep- and the shallow-rooted genotypes did not differ in root hair length, and that RIL 7 had a higher specific root length than RIL 38. Nevertheless, even if some physiological or morphological differences existed, they would not have been large enough to affect the growth rate, which remained similar between both RILs in solution culture. This suggests that no difference existed between both genotypes in their capability for phosphorus uptake or biomass production. Overall, results from the

**Table 7. Average values and standard errors (between brackets) for several above and belowground parameters in the hydroponics experiment. Factors were phosphorus (low and high) and genotype (RILs 7 and 38).**

| | Low phosphorus | | High phosphorus | |
|---|---|---|---|---|
| | RIL 7 | RIL 38 | RIL 7 | RIL38 |
| Leaf area ($cm^2$) | 126.41 (15.10) | 117.21 (10.86) | 631.04 (56.15) | 669 (50.12) |
| Root biomass (g) | 0.21 (0.03) | 0.18 (0.02) | 0.61 (0.05) | 0.43 (0.04) |
| Total biomass (g) | 0.79 (0.10) | 0.73 (0.07) | 3.10 (0.29) | 2.66 (0.22) |
| Root length (m) | 89.0 (7.8) | 39.4 (6.3) | 113.6 (7.2) | 53.2 (8.8) |
| Root/shoot | 0.37 (0.03) | 0.33 (0.02) | 0.24 (0.02) | 0.19 (0.01) |
| Specific root length (m/g) | 459 (6.25) | 216 (2.04) | 198 (2.22) | 121 (1.02) |

solution culture experiment indicate that the differences in the belowground plant parameters observed between RILs in the solid media experiments (basically root gravitropism) would have played an important role in defining the differences found in biomass accumulation.

Our results are consistent with both of our initial hypotheses. In the field site in Guangzhou, greater phosphorus availability toward the soil surface was observed in both phosphorus treatments. This indicates that the shallow-rooted genotypes would have had an advantage in acquiring the limiting resource not only in the fertilized soil but also in the non-fertilized soil. The shallow-rooted RILs were more productive in the field than the deep-rooted ones. Differences between RILs were larger when contrasting RILs were grown in competition. The shallow-rooted RILs were strongly favored by competition. Shoot biomass of these RILs almost doubled the deep-rooted ones when in competition. This greater competitiveness was reflected by the competitive index. The benefit of having a shallow root system was corroborated in the solid culture experiment, where three treatments representing different soil phosphorus spatial distribution and concentration were compared. Root shallowness did not confer any growth advantage if the media was homogeneous, but when phosphorus was concentrated in the topsoil, shallow rooted genotypes had a growth advantage.

Geometric modeling in SimRoot showed that basal root gravitropism could substantially affect competition for phosphorus between adjacent bean root systems because of colocalization of root foraging and phosphorus availability, as well as reduced competition among roots of a shallow-rooted plant (Rubio et al., 2001). Our present results support modeling predictions. Possible disadvantages to shallow rooted genotypes include poorer anchorage and reduced water acquisition. Anchorage did not appear to be different in these RILs in the field, perhaps because of the dominant role of the taproot in this function (Ennos and Fitter, 1992). In the case of water, it should be noted that South China has a humid climate and that during the course of the field work water availability was not a limiting factor. In the case of soils which are prone to drought as well as nutrient deficiency, it is possible that cooptimization of root architecture for multiple constraints is possible through architectural plasticity or complementarity of diverse parts of the root system, notably between basal roots and adventitious roots (Lynch and Brown, 2001)

Belowground competition appears to be more complex than aboveground competition since it involves water and 16 essential nutrients, whose availability is affected by many distinct factors (Casper and Jackson, 1997). Instead, aboveground competition only comprises one resource, light, which explains why shading among neighboring plants is by far the principal factor controlling aboveground competitive ability. Shoot architecture is an important factor in light competition, because it determines leaf deployment throughout a canopy (Grime, 1979; Enquist and Niklas, 2002). Similarly, root architecture could enable plants to be superior competitors for belowground resources if spatial coincidence exists between the limiting resource and the soil volumes explored by roots. In this paper we show evidence that a specific root architecture, with more roots in the top layers of the soil, may lead to success in soils in which the limiting resource for plant growth is concentrated in the upper horizons. This is the case of common bean growing in fields of southern China, in which phosphorus is a limiting factor and water is not, since the periodic rainfall events keep the upper layers of the soil wet. Our observations are relevant to bean breeding, since in developing countries bean is commonly grown in polygenetic stands of mixed species and/or mixed genotypes (Pilbeam et al., 1994; Rezende and Ramalho, 1994).

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