

Phene Synergism between Root Hair Length and Basal Root Growth Angle for Phosphorus Acquisition¹[OPEN]

Magalhaes Amade Miguel², Johannes Auke Postma³, and Jonathan Paul Lynch*

Department of Plant Science, Pennsylvania State University, University Park, Pennsylvania 16802

ORCID IDs: 0000-0002-5222-6648 (J.A.P.); 0000-0002-7265-9790 (J.P.L.).

Shallow basal root growth angle (BRGA) increases phosphorus acquisition efficiency by enhancing topsoil foraging because in most soils, phosphorus is concentrated in the topsoil. Root hair length and density (RHL/D) increase phosphorus acquisition by expanding the soil volume subject to phosphorus depletion through diffusion. We hypothesized that shallow BRGA and large RHL/D are synergetic for phosphorus acquisition, meaning that their combined effect is greater than the sum of their individual effects. To evaluate this hypothesis, phosphorus acquisition in the field in Mozambique was compared among recombinant inbred lines of common bean (*Phaseolus vulgaris*) having four distinct root phenotypes: long root hairs and shallow basal roots, long root hairs and deep basal roots, short root hairs and shallow basal roots, and short root hairs and deep basal roots. The results revealed substantial synergism between BRGA and RHL/D. Compared with short-haired, deep-rooted phenotypes, long root hairs increased shoot biomass under phosphorus stress by 89%, while shallow roots increased shoot biomass by 58%. Genotypes with both long root hairs and shallow roots had 298% greater biomass accumulation than short-haired, deep-rooted phenotypes. Therefore, the utility of shallow basal roots and long root hairs for phosphorus acquisition in combination is twice as large as their additive effects. We conclude that the anatomical phene of long, dense root hairs and the architectural phene of shallower basal root growth are synergetic for phosphorus acquisition. Phene synergism may be common in plant biology and can have substantial importance for plant fitness, as shown here.

Suboptimal phosphorus availability is a primary limitation to plant growth in terrestrial ecosystems (Vance et al., 2003). Large areas of tropical and subtropical soils in Africa, Latin America, and Asia have phosphorus availability limited by low total phosphorus content as well as high phosphorus fixation (Sanchez and Uehara, 1980). The use of phosphorus fertilizer to correct phosphorus deficiency is only a partial solution, since phosphorus fertilizers are costly, nonrenewable, potentially harmful to the environment, and often marginally effective in tropical soils because of immobilization by the soil (Cathcart, 1980). Therefore, the development of crop cultivars with enhanced ability to acquire phosphorus is an important strategy to increase agricultural productivity in low-input agroecosystems and to reduce input requirements in intensive agriculture (Vance et al., 2003;

Gahoonia and Nielsen, 2004; Lambers et al., 2006; Lynch, 2007, 2011).

Several root phenes (i.e. basic units of the phenotype; Serebrovsky, 1925; Lynch, 2011; for discussion, see York et al., 2013) enhance phosphorus acquisition, including root architectural phenes for topsoil foraging (Lynch and Brown, 2001), such as shallow root growth angles (Liao et al., 2004; Ho et al., 2005), increased basal root whorl number (Lynch and Brown, 2012; Miguel et al., 2013), and adventitious rooting (Miller et al., 2003); phenes to enhance soil exploitation, including root hair length and density (RHL/D; Bates and Lynch, 2000a, 2000b, 2001; Ma et al., 2001a; Gahoonia and Nielsen, 2004; Yan et al., 2004) and phosphorus-solubilizing root exudates (Ryan et al., 2001); mycorrhizal symbioses (Smith and Read, 2008); and phenes that reduce the metabolic cost of soil exploration (Lynch and Ho, 2005), such as root etiolation and root cortical aerenchyma (Fan et al., 2003; Postma and Lynch, 2010, 2011). It is probable that interactions among these phenes are important in determining the phosphorus acquisition of integrated phenotypes. Results from the structural-functional model *SimRoot* indicate that RHL/D, the distance from the root tip to the first appearance of root hairs, and the pattern of root hair-bearing epidermal cells (trichoblasts) among non-hair-bearing cells (atrachoblasts) are synergetic for phosphorus acquisition in *Arabidopsis thaliana* (Ma et al., 2001b). Another *SimRoot* study showed that on low-phosphorus soils, the utility of root cortical aerenchyma in maize (*Zea mays*) may be 2.9 times greater in plants with increased lateral branching density than in plants with normal branching (Postma and Lynch, 2011).

¹ This work was supported by the McKnight Foundation Collaborative Crop Research Program and the U.S. Agency for International Development (Pulse Collaborative Crop Research Program and Feed the Future Innovation Laboratory for Climate Resilient Beans, project no. AID-OAA-A-13-00077).

² Present address: Governo da Provincia de Manica, Instituto de Investigação Agrária de Moçambique, Centro Zonal Centro, Estação Agrária de Sussundenga, Postal Code 42, Chimoio, Manica, Mozambique.

³ Present address: Forschungszentrum Jülich, IBG-2: Plant Sciences, 52425 Jülich, Germany.

* Address correspondence to jpl4@psu.edu.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Jonathan Paul Lynch (jpl4@psu.edu).

[OPEN] Articles can be viewed without a subscription.

www.plantphysiol.org/cgi/doi/10.1104/pp.15.00145

Morphological, anatomical, symbiotic, and biochemical phenes expressed by root axes should have significant synergies with architectural phenes, since architectural phenes determine the position of root axes in time and space and, therefore, the soil domain in which spatially localized phenes are expressed (Lynch, 2011).

Phosphorus availability is greater in the topsoil, with a steep decline with depth. Therefore, root architectural phenes that increase topsoil foraging can improve phosphorus acquisition (Lynch and Brown, 2001). Root shallowness regulated by basal root growth angle (BRGA) has been demonstrated to be of particular importance for topsoil foraging (Bonser et al., 1996; Liao et al., 2001; Rubio et al., 2001; Ho et al., 2005). These studies show that common bean (*Phaseolus vulgaris*) genotypes with smaller BRGA (i.e. shallower roots) have better performance in low-phosphorus soils. Shallow root distribution is also important for phosphorus acquisition in maize (Zhu et al., 2005).

RHL/D are also important for phosphorus acquisition (Bates and Lynch, 2000a, 2000b, 2001; Gahoonia and Nielsen, 2004). Since phosphorus mobility in soil is governed by diffusion rather than mass flow, phosphorus uptake by roots is limited by localized phosphorus depletion in the rhizosphere (Barber, 1995). Long root hairs extend the phosphorus depletion zone surrounding the root, thereby increasing the total amount of phosphorus accessible by the roots and phosphorus acquisition. In many plant species, the length and density of root hairs increase in response to low phosphorus availability (Bates and Lynch, 1996; Ma et al., 2001a). Increased RHL/D increases phosphorus accumulation in *Arabidopsis* growing in low-phosphorus conditions (Bates and Lynch, 2000a, 2000b), and mutants lacking root hairs have reduced phosphorus acquisition (Bates and Lynch, 2000b; Gahoonia et al., 2001). Species that develop more and/or longer root hairs (e.g. *Lolium perenne*) are more efficient in accessing inorganic phosphorus from soils and thus show greater growth response to phosphorus fertilization than species that lack these traits (e.g. *Podocarpus totara*). Genotypic variation for root hairs is associated with increased phosphorus acquisition in several species, including barley (*Hordeum vulgare*; Gahoonia and Nielsen, 2004), common bean (Miguel, 2004; Yan et al., 2004), and maize (Zhu et al., 2010).

We hypothesize that the utilities of BRGA and RHL/D for phosphorus acquisition are synergistic. Root hairs will be more valuable for phosphorus acquisition if located in surface soil horizons by arising from roots with a shallow growth angle; shallow roots will have greater benefit for phosphorus acquisition if they have long and dense hairs. Therefore, genotypes possessing long, dense root hairs on shallow roots should have greater phosphorus acquisition than genotypes with either long root hairs on deep roots or short root hairs on shallow roots. We expect the combined benefit of long root hairs and shallow root growth angles to exceed the sum of their individual effects, since they permit greater exploitation of soil strata with the greatest phosphorus availability.

In this study, we evaluated the potential synergism between the architectural phene of BRGA and the morphological phene of RHL/D for phosphorus acquisition by comparison of contrasting phenotypes of common bean growing in a weathered tropical soil.

RESULTS

Analysis of the soil samples taken in the experimental plots after harvest showed stratified phosphorus in both low- and medium-phosphorus plots, with the top 10 cm containing three times more extractable phosphorus than deeper strata (Supplemental Fig. S1).

Under low phosphorus, the three long-shallow genotypes had the best performance in terms of shoot dry weight, phosphorus accumulation, and total leaf area (Fig. 1; Supplemental Figs. S2 and S3). The long-deep and short-shallow phenotypes had intermediate performance under low phosphorus. These two categories did not differ significantly for shoot dry weight, phosphorus content, or total leaf area per plant. Short-deep phenotypes had the least shoot biomass, tissue phosphorus content per plant, and total leaf area.

Shoot biomass accumulation in the long-shallow phenotypes was significantly greater ($P < 0.05$) than the sum of biomass increase attributed to the individual root hair and angle traits (Table II; Fig. 1). A positive interaction effect also was found for total phosphorus uptake, total leaf area, root length density at 0 to 15 cm, and the number of adventitious roots (Table I).

Under medium phosphorus availability (i.e. treatments with applied phosphorus fertilizer but still experiencing growth reduction due to suboptimal phosphorus availability), the long-shallow phenotype had greater shoot biomass and tissue phosphorus content than the other

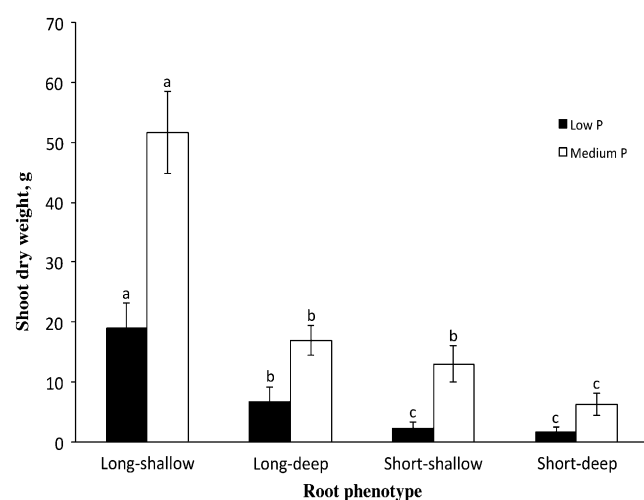


Figure 1. Shoot dry weight of four phenotypes grown under low and medium phosphorus availability in the field in Mozambique. Each mean is from four replicates of three genotypes. Error bars represent SE. Means followed by the same letter within each phosphorus level are not statistically different.

Table 1. Results of linear models comparing specific treatments and the effects of various growth parameters

Phosphorus level indicates soil phosphorus availability: medium (18 mg L⁻¹) and low (5 mg L⁻¹); BRGA is measured in ° from horizontal; RHL is measured in ° from horizontal; RHL is measured in mm. df, Degrees of freedom. *, Significant at P ≤ 0.05; **, significant at P ≤ 0.01; ***, significant at P ≤ 0.001.

| Effect | Shoot Dry Weight | | P Content | | Total Leaf Area | | Basal Root Growth Length | | RHL | | Adventitious Roots | | Root Length at 0- to 15-cm Depth | | Root Length at 15- to 30-cm Depth | | Total Core Root Length | | No. of Adventitious Roots | | |
|-------------------------------|------------------|-----------|-----------|-----------|-----------------|-----------|--------------------------|-----------|------|-----------|--------------------|----------|----------------------------------|-----------|-----------------------------------|-----------|------------------------|-----------|---------------------------|----------|----|
| | df | F Ratio | df | F Ratio | df | F Ratio | df | F Ratio | df | F Ratio | df | F Ratio | df | F Ratio | df | F Ratio | df | F Ratio | df | F Ratio | |
| Phosphorus level | 1 | 81.62*** | 1 | 157.49*** | 1 | 66.84*** | 1 | 59.31*** | 1 | 86.90*** | 1 | 0.01 | 1 | 274.19*** | 1 | 126.88*** | 1 | 321.32*** | 1 | 0.01 | |
| BRGA | 1 | 72.75*** | 1 | 620.16*** | 1 | 62.69*** | 1 | 523.45*** | 1 | 21.58*** | 1 | 0.00 | 1 | 17.49*** | 1 | 335.27*** | 1 | 90.48*** | 1 | 0.00 | |
| RHL | 1 | 112.46*** | 1 | 817.55*** | 1 | 111.23*** | 1 | 11.00*** | 1 | 713.34*** | 1 | 0.07 | 1 | 23.15*** | 1 | 1.23 | 1 | 14.24*** | 1 | 0.07 | |
| BRGA × RHL | 1 | 45.53*** | 1 | 197.23*** | 1 | 39.75*** | 1 | 13.35*** | 1 | 0.27 | 1 | 2.77 | 1 | 35.09*** | 1 | 3.42 | 1 | 24.66*** | 1 | 2.77** | |
| Phosphorus level × BRGA | 1 | 28.86*** | 1 | 12.32*** | 1 | 3.62** | 1 | 2.66 | 1 | 0.38 | 1 | 0.88 | 1 | 0.69 | 1 | 5.02** | 1 | 4.05 | 1 | 0.88 | |
| Phosphorus level × RHL | 1 | 27.51*** | 1 | 0.24 | 1 | 27.39*** | 1 | 1.39 | 1 | 5.25** | 1 | 15.23*** | 1 | 0.23 | 1 | 21.45*** | 1 | 7.68** | 1 | 15.23*** | |
| Phosphorus level × BRGA × RHL | 1 | 14.79*** | 1 | 1.15 | 1 | 29.77*** | 1 | 1.98 | 1 | 0.30 | 1 | 0.33 | 1 | 0.02 | 1 | 0.17 | 1 | 0.14 | 1 | 0.33 | |
| Error | 87 | | 88 | | 88 | | 88 | | 88 | | 88 | | 88 | | 88 | | 88 | | 88 | | 88 |
| Total | 94 | | 95 | | 95 | | 95 | | 95 | | 95 | | 95 | | 95 | | 95 | | 95 | | 95 |
| F ² | 0.49 | | 0.86 | | 0.497 | | 0.78 | | 0.79 | | | | | | | | | | | | |

phenotypes (Table II). The long-deep and short-shallow phenotypes did not differ when grown under medium phosphorus availability (Fig. 1; Table II; Supplemental Fig. S2). Genotypes with shallow BRGA had greater biomass and greater tissue phosphorus content compared with genotypes with greater BRGA (Fig. 1; Supplemental Fig. S2). Long-shallow phenotypes had significantly greater leaf area compared with other phenotypes under both low and medium phosphorus availability (Supplemental Fig. S2). Long-deep and short-deep genotypes showed greater basal root angles compared with long-shallow and short-shallow genotypes (Fig. 2). Synergism also was observed in relation to shoot biomass. Large RHL/D and shallow BRGA both increased shoot biomass. When long root hairs were on shallow roots, the increase in shoot biomass was greater than the sum of biomass increase that resulted from the two root traits appearing separately (Table II).

Under low phosphorus availability, long root hairs increased phosphorus acquisition by 2.78 mg per plant compared with the baseline (Supplemental Table S1). Shallow basal roots increased phosphorus acquisition by 2.42 mg per plant compared with the baseline (Supplemental Table S1). Thus, the additive effect on phosphorus acquisition of both phenes would be an additional 5.2 mg per plant. Genotypes possessing both long hairs and shallow basal roots actually had additional phosphorus accumulation of 8.13 mg per plant, which is 2.93 mg per plant more phosphorus than expected from the additive effects, which is the total effect: that is, the independent phene effect plus the synergistic effect (Supplemental Table S1). Although smaller than in low-phosphorus treatments, the synergistic effect also was observed in plants growing under medium phosphorus availability (298.9% versus 87.7%).

Genotypes used in this study varied for root hair length and BRGA. Data from plants taken at 14 DAP confirmed the variation in root phenotypes of the genotypes assigned to each phenotypic category (Figs. 2 and 3). Long-shallow and short-shallow phenotypes had BRGA ranging from less than 10° to 40° from horizontal. Long-deep and short-deep phenotypes had greater BRGA, varying from 60° to 80° from horizontal. Overall, these genotypes had about 10° steeper angles under medium phosphorus availability compared with low phosphorus availability. Under low phosphorus, short-shallow genotypes had significantly greater BRGA than long-shallow genotypes. This difference is small compared with the overall differences between the deep and shallow genotypes but could mean that we slightly underestimated the synergistic effect of having both shallow and short root hairs. Similar to the plasticity responses in BRGA, we observed plasticity in RHL. RHL was about 0.1 mm longer in low-phosphorus plots. On medium-phosphorus plots, short-shallow genotypes had significantly greater RHL than short-deep genotypes. This difference was much smaller than the difference between the long and short genotypes, which could mean that we slightly overestimated the effects of BRGA. In addition to the RHL and BRGA, genotypes were

Table II. Percentage increase of shoot dry weight in 12 common bean genotypes grouped in the following phenotypes: long root hairs and shallow roots; long root hairs and deep roots; short root hairs and shallow roots; and short root hairs and deep roots

Plants were grown with low and medium phosphorus in the field. Plant samples were collected at 28 d after planting (DAP). Each value in shoot dry weight (SDW) of a genotype is a mean value of four replicates. Percentages were calculated from the average shoot dry weight of phenotype D (8.88 g = 100% for low phosphorus and 22.66 g = 100% for medium phosphorus). The following formula was used to calculate the percentage of SDW increase: % X = (average SDW of X × 100)/(average SDW of D) – 100%, where X = phenotype. Means followed by different letters are significantly different at $P \leq 0.05$.

| Phenotype | Genotype | Phosphorus Treatment | | | | | |
|---------------|----------|----------------------|-------------|----------|-------------------|-------------|----------|
| | | Low Phosphorus | | | Medium Phosphorus | | |
| | | SDW | Average SDW | Increase | SDW | Average SDW | Increase |
| | | | g | % | | g | % |
| Long shallow | DG53 | 37.38 | | | 42.50 | | |
| | DG38 | 35.11 | | | 45.23 | | |
| | DG13 | 33.78 | 35.42a | 298.9 | 39.90 | 42.54a | 87.7 |
| Long deep | DG32 | 17.23 | | | 27.36 | | |
| | DG79 | 16.63 | | | 30.75 | | |
| | DG52 | 16.56 | 16.81b | 89.3 | 26.65 | 28.25b | 24.7 |
| Short shallow | DG36 | 15.54 | | | 25.67 | | |
| | DG66 | 13.70 | | | 23.81 | | |
| | DG64 | 12.78 | 14.01b | 57.7 | 22.90 | 24.13b | 6.5 |
| Short deep | DG47 | 10.01 | | | 20.14 | | |
| | DG19 | 8.89 | | | 24.98 | | |
| | DG27 | 7.74 | 8.88c | 0 | 22.86 | 22.66b | 0 |

evaluated for the total number of adventitious roots, as adventitious root formation is known to be a beneficial trait for phosphorus uptake and plant growth on low-phosphorus soils (Miller et al., 2003). On average, genotypes did not differ in the number of adventitious roots, but on low-phosphorus soils, short-deep genotypes had significantly more adventitious roots than long-deep genotypes. This could mean that we slightly underestimated the effect of root hairs on phosphorus uptake and shoot growth on low-phosphorus soils (Table I; Supplemental Fig. S4). Under low phosphorus availability, long-shallow genotypes had statistically greater growth rate at 21 to 28 DAP compared with the rest of the phenotypes (Table III). In addition, short-deep genotypes had slower growth rates than the three other categories, which were not statistically different among them. Under medium phosphorus, long-shallow genotypes had statistically greater growth rates than the other root phenotype categories. Growth rates of the remaining three root phenotypes (long deep, short shallow, and short deep) were not statistically different ($P = 0.05$) among them.

Root cores were taken at 28 DAP at two soil depths, 0 to 15 cm and 15 to 30 cm, along planting rows. Genotypes had greater root length in cores taken in medium-phosphorus treatments compared with low-phosphorus treatments (Fig. 4; Table I). Root length in root cores taken at 0 to 15 cm did not differ significantly among long-shallow, long-deep, and short-shallow phenotypes under either low or medium phosphorus availability. The short-shallow phenotype had less root length than the other phenotypes (Fig. 4, top). At 15 to 30 cm, the long-deep and short-deep phenotypes had greater root length than the long-shallow and short-shallow phenotypes

(Fig. 4, bottom). Under low phosphorus, the long-shallow phenotype had significantly less root length in the deeper 15- to 30-cm layer, in agreement with its shallower BRGA (Figs. 2 and 4, bottom).

Since root length in soil cores taken from 0 to 15 cm were not significantly different among phenotypes, we hypothesized that this was caused by the fact that the root cores were taken very close to the plant, which may have obscured root distributional differences farther away from the stem. To demonstrate this hypothesis, we simulated the root distribution in virtual cores centered

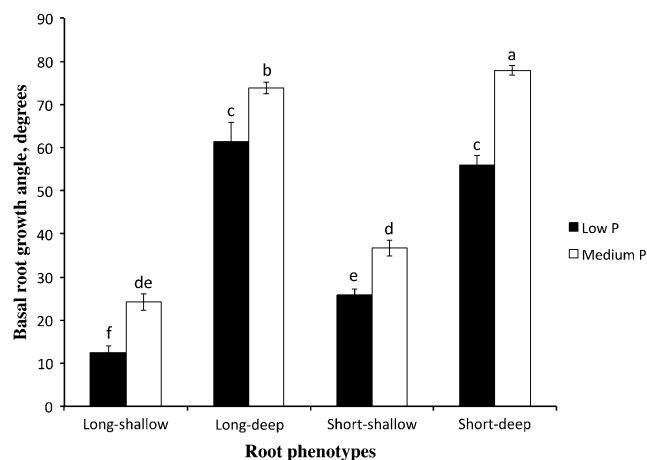


Figure 2. BRGA of 12 genotypes grouped according to their root phenotypes grown under low phosphorus and medium phosphorus availability in the field. Each mean is from four replicates of three genotypes. Error bars represent \pm SE. Means followed by the same letter are not statistically different.

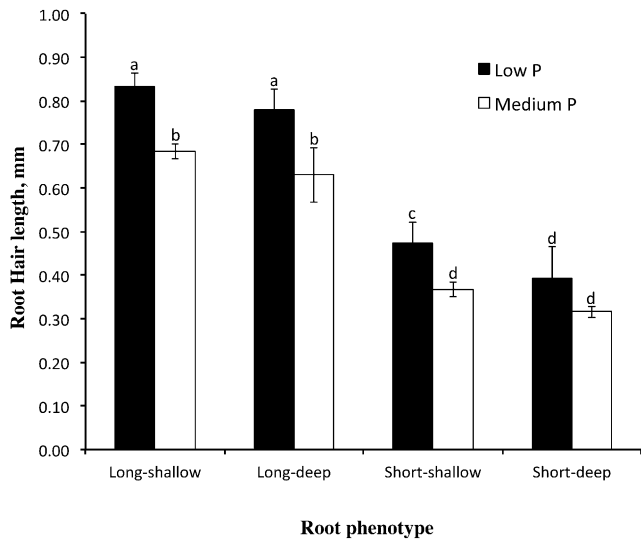


Figure 3. RHL of four root phenotypes of common bean grown under low and medium phosphorus availability in the field. Each mean is from four replicates of three genotypes. Error bars represent se. Means followed by the same letter are not statistically different.

either 5 or 15 cm from the stems of shallow- and deep-rooting bean plants at 28 d after germination and growing with the same (inter)row spacing (Fig. 5). Simulation results showed statistically significant differences between shallow-rooted and deep-rooted genotypes at 0- to 15-cm depth with samples taken 15 cm away from the planting row (Fig. 5, top). These differences were not observed in actual root cores taken at 5 cm away from the plant. Soil coring at 10-cm depth intervals shows that shallow-rooted genotypes allocate more roots to the topsoil, decreasing with depth, while deep-rooted genotypes increased the density of root deployment with depth, with a sudden decrease at 40 to 50 cm (Fig. 5, bottom). Root density decreased with depth in shallow-rooted phenotypes and increased with depth in deep-rooted genotypes with the pick at 40-cm depth (Supplemental Fig. S5). Significant differences in root allocation by depth were observed among phenotypes in simulated cores taken at 15 cm away from the plant, between the planting rows (Fig. 6).

The study was repeated in 2014 with the same genotypes and field sites, with similar results: BRGA and RHL/D showed substantial positive synergism

for plant growth and pod yield under medium- and low-phosphorus conditions (Supplemental Figs. S6 and S7; Supplemental Table S2).

DISCUSSION

Our results confirm that both BRGA and RHL/D are beneficial for phosphorus acquisition in isolation, in agreement with previous studies (see refs. cited above). Our results also support the hypothesis that BRGA and RHL/D are synergetic for phosphorus accumulation. Under low phosphorus, larger RHL/D increased shoot growth 89.3% while shallow BRGA increased shoot growth by 57.7%, compared with the short-deep phenotype. The combination of large RHL/D and shallow BRGA improved shoot growth by 298%, double that expected if the growth effects of these traits did not interact. Under low phosphorus, larger RHL/D increased phosphorus content 90% while shallow BRGA increased phosphorus content by 79%, compared with the short-deep phenotype. The combination of large RHL/D and shallow BRGA improved phosphorus content by 265%, double that expected from an additive effect (Supplemental Table S1).

As expected, RHL/D and BRGA increased growth on medium-phosphorus plots to a lesser extent than they improved growth on the low-phosphorus plots. RHL/D increased growth by 25% and BRGA by 7% under medium phosphorus availability. Nevertheless, the combined phenotype increased growth by 88%. This is nearly three times the sum (25 + 7 = 32). Therefore, the synergism might be greater on medium-phosphorus soils than on low-phosphorus soils. We do not currently have a mechanistic explanation for the observed differences between low- and medium-phosphorus soils, but part of the explanation may come from the growth rate data, which show that the synergism on medium-phosphorus plots is already strongly observed after 21 d, while the synergism on the low-phosphorus plots is only observed after 28 d. This might be due to the fact that root growth under low phosphorus takes longer to reach a certain length than under medium phosphorus. As a result, the synergetic effect could be observed later. The result at medium phosphorus also suggests that the benefit of shallow BRGA or greater RHL/D might go undetected without the expression of the other phene (Fig. 1). This clearly demonstrates that univariate

Table III. Growth analysis of four phenotypes grown under low and medium phosphorus availability

G_R = growth rates at a given period, expressed in g of shoot dry weight per week. Values followed by the same letter in a column are not statistically different. Each value is a mean from four replicates. Significance (Signif.) is at 5% probability. W, Dry weight; t, time.

| Phene | Mean Log of Shoot Dry Weight, Low Phosphorus | | | Mean Growth Rates (log dW/dt), Low Phosphorus | | | | Mean Log of Shoot Dry Weight, Medium Phosphorus | | | Mean Growth Rates (log dW/dt), Medium Phosphorus | | | |
|---------------|--|--------|--------|---|---------|---------------------|---------|---|--------|--------|--|---------|---------------------|---------|
| | 14 DAP | 21 DAP | 28 DAP | G _{R14-21} | Signif. | G _{R21-28} | Signif. | 14 DAP | 21 DAP | 28 DAP | G _{R14-21} | Signif. | G _{R21-28} | Signif. |
| Long shallow | 1.04 | 1.30 | 1.72 | 0.037 | a | 0.060 | a | 1.19 | 1.56 | 1.88 | 0.045 | a | 0.046 | a |
| Long deep | 0.90 | 1.04 | 1.30 | 0.051 | b | 0.037 | b | 1.11 | 1.36 | 1.76 | 0.036 | a | 0.057 | a |
| Short shallow | 0.84 | 1.15 | 1.45 | 0.044 | ab | 0.042 | b | 1.05 | 1.32 | 1.72 | 0.038 | a | 0.057 | a |
| Short deep | 0.70 | 1.26 | 1.48 | 0.049 | ab | 0.031 | c | 1.00 | 1.22 | 1.58 | 0.031 | a | 0.051 | a |

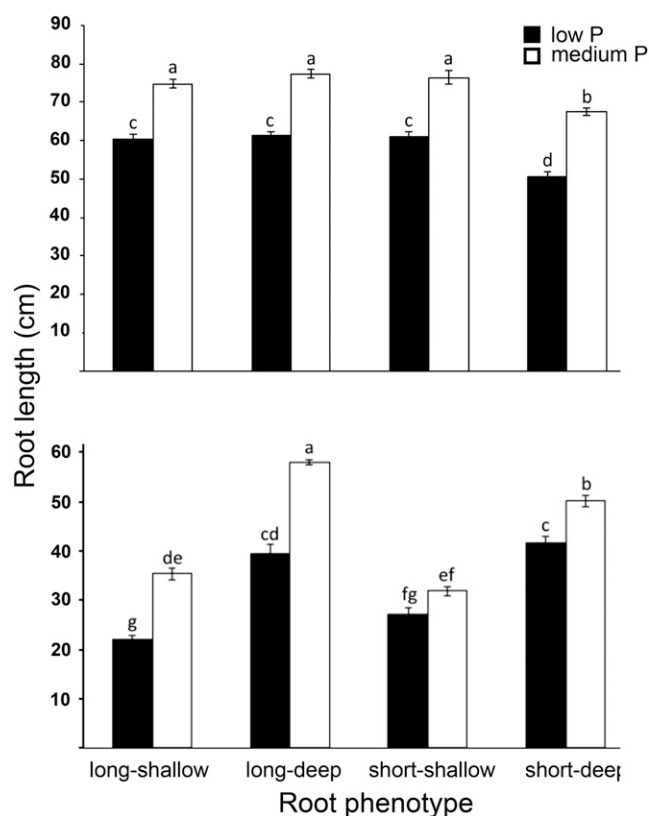


Figure 4. Root length in 0- to 15-cm (top) and 15- to 30-cm (bottom) soil strata of four phenotypes grown under low and medium phosphorus availability in the field in Mozambique. Each mean is from four replicates of three genotypes. Error bars represent SE. Means followed by the same letter are not statistically different.

analyses in which growth performance on low-fertility soils is correlated with single quantitative traits or loci might not always reveal the utility of a phene or a quantitative trait locus, and multivariate analyses are required if we are to understand how different phenes interact. A complicating factor in root studies is that root phenotypes may vary across studies or environmental conditions. Although we did observe some plasticity in response to phosphorus in the RHL/D and BRGA phenotypes, the plasticity responses were much smaller than the difference between the groups of genotypes representing contrasting phenotypes. Shallow BRGA genotypes were shallow in both the field and the laboratory, and the same was true for long RHL/D genotypes. We conclude that early seedling screens for RHL/D and BRGA can be effectively employed in selection programs.

Shallow BRGA is thought to increase phosphorus uptake by placing relatively more roots in shallow soil layers, where phosphorus availability is greater, at the cost of placing fewer roots in deep soil strata (Ho et al., 2005; Basu et al., 2007). Although we could confirm that the shallow BRGA phenotypes had fewer deep roots, we did not observe greater root length in the top 15 cm, as we had expected. We explain this by taking the

horizontal dimension into account. We suggest that shallow versus deep rooting is more easily measured between the rows than close to the plant, as we did in this study. This is so because even deep-angled genotypes have their basal roots in shallow soil very close to the hypocotyl from which they originate. We used the functional structural model *SimRoot* to illustrate this idea and quantify the effect on root distribution in a virtual core; we show that our observations of root length distribution in the core are not in disagreement with the assumption that the shallow BRGA genotypes indeed placed more roots in the 0- to 15-cm zone (Supplemental Fig. S5).

In order to strengthen our thesis that observed growth responses can be attributed to the individual phenes and the synergism between them, we confirmed that the RHL/D and BRGA phenotypes of the inbred lines we employed were stable across the laboratory and field environments, and we also checked that we did not introduce a bias by having differences in adventitious root formation. Adventitious root formation has been shown

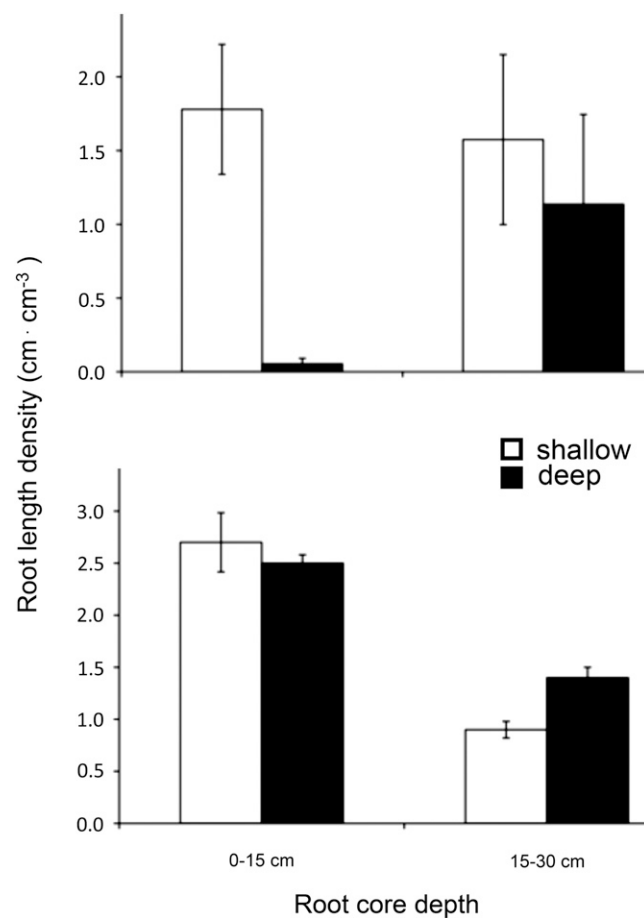


Figure 5. Simulated root density of root cores taken at 0- to 15-cm depth and at 15 cm away from the planting row (top) and root cores taken at 0- to 15-cm depth and at 5 cm within the planting row (bottom) of common bean genotypes. Genotypes were contrasting for BRGA; root core samples were taken at 28 DAP under low phosphorus ($5 \mu\text{g g}^{-1}$) availability. Each mean is from four simulated replicates.

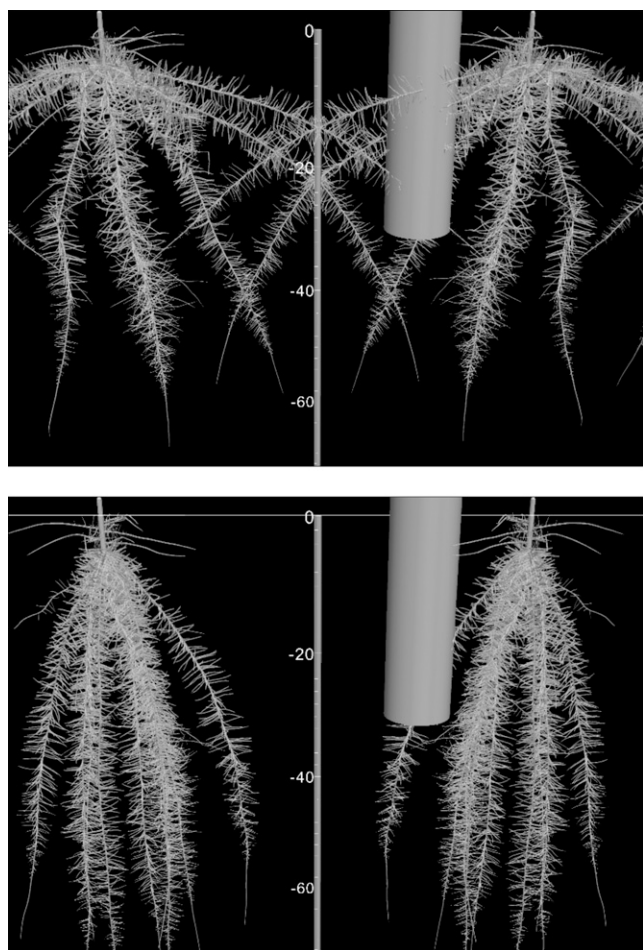


Figure 6. Graphic representation of root coring between planting rows in shallow-rooted genotypes (top) and deep-rooted genotypes (bottom), at 28 DAP, under low phosphorus availability. The coring cylinder has a 10-cm diameter.

to increase phosphorus uptake on low-phosphorus soils (Miller et al., 2003). However, we confirmed that our genotypes were very similar with respect to this trait.

BRGA is thought to increase phosphorus acquisition by positioning relatively more roots in shallow soil domains where phosphorus availability is greater. It thus effectively increases the initial concentration in the phosphorus depletion zones. In contrast, RHL/D increases the potential size of the depletion zones. If the potential uptake of phosphorus is mostly determined by the volume of soil explored multiplied by the bioavailability of phosphorus in that volume, this provides a logical explanation for the observed synergism. This would mean that the effects of BRGA and RHL/D are multiplicative. Although this seems to be the case on low-phosphorus soils ($1.58 \times 1.89 \times 100 = 299\%$; compare with the observed 293%), the effects on the medium-phosphorus soil are far greater than multiplicative. This demonstrates that our explanation, although logical, might be oversimplified, not taking feedbacks such as increased root growth (positive feedback) or root

competition (negative feedback) into account. Nevertheless, it provides a mechanistic explanation for the observed phene synergism.

Relatively few studies have addressed interactions among phenes associated with soil resource acquisition in plants, despite the importance of these interactions for plant fitness (York et al., 2013). Computer simulations with the functional-structural model *SimRoot* showed substantial synergism among four root hair phenes (length, density, initiation, and geometry) for phosphorus acquisition in *Arabidopsis* (Ma et al., 2001b). Another *SimRoot* study indicated that root cortical aerenchyma may be synergistic with lateral branching frequency for phosphorus acquisition in maize (Postma and Lynch, 2011). To our knowledge, this study is the first to empirically demonstrate synergism between architectural and anatomical phenes for soil resource acquisition.

Functional interactions are probably common and important for root phenes (York et al., 2013). An array of root phenes are involved in phosphorus acquisition, including phenes affecting soil exploration through root growth and architecture, phenes for fine-scale soil exploitation such as root hairs, phenes affecting the mobilization of phosphate from sparingly soluble forms in the rhizosphere, and phenes for symbioses with mycorrhizal fungi and phosphorus-solubilizing microbes in the rhizosphere (Lynch and Brown, 2008). Root architecture has an important overarching role in positioning root axes in soil domains with the greatest phosphorus availability and, therefore, should display significant synergisms with phenes operating on finer spatial scales, as shown in this study. We hypothesize that significant synergisms also should occur between architectural phenes and root exudates as well as mycorrhizal symbioses. Phosphatase exudates will be more useful in soil domains with greater content of organic phosphorus, generally the topsoil. Carboxylate exudates may be more useful in subsurface horizons in which phosphorus is bound to iron and aluminum oxides. Mycorrhizal symbioses should display synergism with architectural phenes, because in natural soil, extraradical hyphae are subject to grazing, so that hyphal foraging for phosphorus is probably concentrated around the root axis, and mycorrhizal fungal propagules are more abundant in shallow soil domains (Lynch and Wojciechowski, 2015). Although phosphorus transport kinetics do not limit phosphorus acquisition from soil because of diffusion limitations, for more mobile resources such as nitrate, we hypothesize that architectural phenes will display synergism with transport kinetics.

Phene interactions also may be driven by competition for internal resources. The synergism between root cortical aerenchyma and lateral branching frequency for phosphorus acquisition in maize is an example of this, since the benefit of reduced root metabolic costs from root cortical aerenchyma is more advantageous in phenotypes with more roots (Postma and Lynch, 2011). Another example is that the benefit of increased adventitious root formation for phosphorus acquisition in common bean may be counteracted by reduced lateral branching of basal roots, an effect driven by root metabolic costs (Walk et al.,

2006). Tradeoffs for external resources also may cause phene interactions, as shown by the example of root growth angles in common bean creating tradeoffs between phosphorus, a shallow resource, and water, a deep resource (Ho et al., 2005). Positive synergies also are possible, such as water acquisition by deep roots increasing nutrient acquisition from dry surface soils via hydraulic lift (Armas et al., 2012).

The importance of phene interactions for soil resource acquisition may be a factor contributing to the wide array of root phenotypes evident within a species (Bayuelo-Jimenez et al., 2011; Burton et al., 2013). This complexity is compounded by the array of soil environments to which plants must adapt, which includes varying resource availabilities in time and space and, in the case of water, substantial stochasticity. Understanding the value of a specific root phene for soil resource acquisition requires an understanding of how fitness is influenced by the phene in an array of potential soil environments and also in the context of an array of root phenotypes (Lynch and Brown, 2012).

MATERIALS AND METHODS

Genotypic Selection

Eighty-six recombinant inbred lines of common bean (*Phaseolus vulgaris*) developed from a cross between DOR364 and G19833 were phenotyped for root traits. Genotype G19833 is a Peruvian landrace of the Andean gene pool with a bush indeterminate growth habit, observed in previous studies as having root characteristics conferring phosphorus acquisition efficiency (Beebe et al., 1997). DOR364 is a high-yielding line with resistance to *Bean golden mosaic virus* developed by the International Center for Tropical Agriculture. Six seeds of each of the recombinant inbred lines were surface sterilized with 6% (v/v) sodium hypochlorite for 5 min, rinsed thoroughly with distilled water, and scarified with a blade. Then, seeds were germinated at 28°C for 2 d in rolled germination paper (25.5 × 37.5 cm; Anchor Paper). The seedlings were then transferred to growth pouches soaked with nutrient solution in low phosphorus [in μM : 3,000 KNO_3 , 2,000 $\text{Ca}(\text{NO}_3)_2$, 250 MgSO_4 , 25 KCl, 12.5 H_3BO_3 , 1 MnSO_4 , 1 ZnSO_4 , 0.25 CuSO_4 , 0.25 $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, 1 KH_2PO_4 , and 25 Fe-Na-EDTA]. Pouches consisted of a sheet of 30- × 24-cm blue germination paper (Anchor Paper) inserted into a polyethylene bag of the same size with aeration holes at 3-cm spacing. Pouches were open at the bottom to allow direct contact with the nutrient solution. Pouches containing seedlings were suspended in nutrient solution at 25°C. BRGA was measured at 28 DAP relative to the horizontal plane (i.e. larger angles indicate steeper BRGA). The range of growth angles for each plant was calculated by subtracting the minimum growth angle from the maximum growth angle exhibited by the basal roots of an individual plant. Genotypes with an average BRGA of less than 45° were considered shallow rooted, whereas genotypes with an average BRGA of greater than 45° were classified as deep rooted. Genotypes were classified according to root hair traits from an analysis of the same population from a previous study (Miguel, 2004). Three genotypes were selected to comprise each of four phenotypes: (1) long, dense root hairs and shallow basal roots; (2) long, dense root hairs and deep basal roots; (3) short, sparse root hairs and shallow basal roots; and (4) short, sparse root hairs and deep basal roots. In total, 12 genotypes were used in the field experiments.

Field Experiments

This study was conducted at the Sussundenga Research Station of the Instituto de Investigacao Agraria de Moçambique in Manica Province (19° 19' 02.00" S, 33° 14' 25.24" E, 620 m above sea level). The soil type at the research site is an Oxisol, Udox suborder, a red loam with low pH (4.5–5.5). Three months before planting, the soils were limed (CaCO_3) to bring the pH to approximately 6.2. The annual average precipitation is 1,100 mm. The rainfall season starts in late October or early November and continues until early April. Temperatures during the growing season ranged from 14°C to 28°C. The experiment was planted on February 1, 2010. Seeds were inoculated with

rhizobia obtained at the Bunda College Microbiology Laboratory shortly before planting. The experiment had medium-phosphorus and low-phosphorus plots. We also applied compound fertilizer and urea (46% [w/w] nitrogen) at 200 kg ha⁻¹ to keep other nutrients at optimal level, with the amount of phosphorus being the only difference in nutrients among the treatments. Simple superphosphate was used as a source of additional phosphorus for medium-phosphorus plots. At harvest, medium-phosphorus plots had 19 ppm and low phosphorus plots had 5.5 ppm phosphorus (Olsen et al., 1954), suggesting that we had moderate-phosphorus and low-phosphorus treatments (Supplemental Fig. S1). Weed control was performed manually. Synthetic pyrethrin insecticide was applied as needed. Sprinkler irrigation was used to maintain soil moisture content near field capacity.

Twelve genotype categories were grown on soils with low (6 ppm) and medium (19 ppm) phosphorus availability (Olsen et al., 1954). Each treatment had four replications in a completely randomized block design. Experimental units were plots, three rows wide and 2 m long; 21 seeds were planted in each row, with spacing of 60 cm between the planting rows and 10 cm within the row (60- × 10-cm spacing).

The study was repeated in 2014 with the same genotypes and conditions.

Data Collection and Analysis

Data collected from the field study included shoot dry weight, leaf area, BRGA, RHL, root distribution with soil depth, and total phosphorus content in shoot tissue. Each plant sampling was performed by taking three representative plants from the middle row. Sampled plants were in the middle of at least three other plants in both directions of the row and bordered with other plants on both sides of the sampling row.

Plant shoots were collected during the growing period of the plants in three harvests. Plant shoot samples were collected at 14, 21, and 28 DAP. During sample collection, shoot samples were washed with dilute bleach. Shoots were dried at 60°C for 5 d for biomass determination. Shoot samples were ashed at 500°C for 15 h and analyzed for phosphorus content spectrophotometrically (Murphy and Riley, 1962).

During shoot sampling, three 7.06-cm² leaf discs per plant were collected from five fully expanded, mature, and active leaves. The leaf discs were dried at 60°C for 5 d for dry weight determination. Specific leaf weight (g cm⁻²) calculated from these samples was used to estimate total leaf area from shoot biomass for each plot.

Root cores were excavated and placed in a 20-L container with detergent in water to loosen soil from the roots. Root samples were then washed and rinsed in clean tap water and placed in snap-cap vials with 25% (v/v) ethanol. Root fragments recovered from each core were scanned, and images were saved for later analysis. The scanned images were analyzed for total root length and root length by root diameter using WinRhizo Pro (Régent Instruments).

For determination of RHL, root fragments were exposed to 0.05% (w/v) Trypan Blue solution for 3 s. Images from the region of the section with a representative root hair phenotype were taken at 40× magnification. A calibration microscale displayed on the screen was used to determine the image size in relation to the actual size at that magnification. Root images were analyzed for RHL using ImageJ software (Wayne Rasband, National Institute of Mental Health). We measured four root fragments per replication (sample), and the average of these four subsamples was used as the value for that sample.

In order to determine root distribution with soil depth, soil cores were taken from the field. Root samples were acquired from soil cores taken within the planting row 5 cm from each plant in each direction. Soil cores were separated into 0- to 15-cm and 15- to 30-cm depths, discarding the remaining length of the soil core. Roots from each core interval were washed and stored in 25% ethanol in water. Total root length from each core interval was determined using WinRhizo Pro (Régent Instruments). BRGA was determined by measurement of excavated root crowns, as described by Trachsel et al. (2011).

Growth analysis was calculated from logarithmic values of shoot dry weight data taken at 14, 21, and 28 DAP, among the four phenotypes, with replicates for each, in both low-phosphorus and medium-phosphorus treatments, according to Hunt et al. (1982). Results of growth were statistically analyzed for mean comparison using Tukey's test on MiniTab statistical software.

Simulation of Root Distribution with Depth in Relation to Soil-Coring Position

To determine the effect of coring position on root distribution with depth, we used SimRoot version 10 (Postma and Lynch, 2011a), a functional-structural

plant model, to simulate the shallow and deep bean root architecture of plants grown in soils with medium phosphorus availability (approximately 50% growth reduction). At 28 d after germination, we determined for both shallow and deep root architectures the root length density (cm cm^{-3}) in a vertical core placed 15 cm from the plant toward the neighboring row. We repeated the study four times to average out stochastic effects in the model. In total, we ran eight simulations. We used for our simulation of beans the same parameter set that was published by Postma and Lynch (2011a). To vary the architecture, we adjusted the branching angle and gravitropism of the basal roots of both basal root whorls (Supplemental Table S3). The phosphorus concentration in the soil solution was $6 \mu\text{M}$. The root length density was determined at 5-cm depth intervals up to a depth of 30 cm. For each depth, the root length density was determined by summing up the length of all the segments less than 5 cm away from the depth position and dividing that root length by the volume of a sphere with a radius of 5 cm (Supplemental Table S3).

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Results of soil sample analysis taken in medium-phosphorus and low-phosphorus plots in the field after harvesting the experiments in Mozambique.

Supplemental Figure S2. Phosphorus content of four phenotypes grown under low and high phosphorus availability in the field in Mozambique.

Supplemental Figure S3. Total leaf area of 12 genotypes grown under low phosphorus and high phosphorus availability in the field.

Supplemental Figure S4. Total number of adventitious roots per plant of root phenotypes, grown under low phosphorus and medium phosphorus availability in the field.

Supplemental Figure S5. Simulated root density of root cores taken at 10-cm intervals of soil depth and at 5 cm within the planting row of common genotypes.

Supplemental Figure S6. Total number of pods of four contrasting root phenotypes grown in the field in Mozambique during the 2014 growing season.

Supplemental Figure S7. Shoot dry weight of four contrasting root phenotypes grown in the field in Mozambique during the 2014 growing season.

Supplemental Table S1. Phene synergism for phosphorus accumulation of genotypes with contrasting RHL/D and basal root growth angle grown under low and high phosphorus availability.

Supplemental Table S2. Percentage of shoot dry weight increase due to synergistic effect of the two root traits in relation to the short deep phenotype.

Supplemental Table S3. Parameters used for simulating deep and shallow bean root architecture.

Received January 30, 2015; accepted February 8, 2015; published February 19, 2015.

LITERATURE CITED

- Armas C, Ordiales R, Pugnaire FI (2012) Measuring plant interactions: a new comparative index. *Ecology* **85**: 2682–2686
- Barber SA (1995) Soil Nutrient Bioavailability: A Mechanistic Approach, Ed 2. John Wiley & Sons, New York
- Basu P, Zhang Y, Lynch JP, Brown KM (2007) Ethylene modulates genetic, positional, and nutritional regulation of root plagiogravitropism. *Funct Plant Biol* **34**: 41–51
- Bates TR, Lynch JP (1996) Stimulation of root hair elongation in *Arabidopsis thaliana* by low phosphorus availability. *Plant Cell Environ* **19**: 529–538
- Bates TR, Lynch JP (2000a) The efficiency of *Arabidopsis thaliana* (Brassicaceae) root hairs in phosphorus acquisition. *Am J Bot* **87**: 964–970
- Bates TR, Lynch JP (2000b) Plant growth and phosphorus accumulation of wild type and two root hair mutants of *Arabidopsis thaliana* (Brassicaceae). *Am J Bot* **87**: 958–963
- Bates TR, Lynch JP (2001) Root hairs confer a competitive advantage under low phosphorus availability. *Plant Soil* **236**: 243–250
- Bayuelo-Jimenez JS, Gallardo-Valdez M, Perez-Decelis VA, Magdaleno-Armas L, Ochoa I, Lynch JP (2011) Genotypic variation for root traits of maize (*Zea mays* L.) from the Purhepecha Plateau under contrasting phosphorus availability. *Field Crops Res* **121**: 350–362
- Beebe S, Lynch JP, Galwey N, Tohme J, Ochoa I (1997) A geographical approach to identify phosphorus-efficient genotypes among landraces and wild ancestors of common bean. *Euphytica* **95**: 325–336
- Bonsler AM, Lynch J, Snapp S (1996) Effect of phosphorus deficiency on growth angle of basal roots in *Phaseolus vulgaris*. *New Phytol* **132**: 281–288
- Burton AL, Brown KM, Lynch JP (2013) Phenotypic diversity of root anatomical and architectural traits in *Zea* species. *Crop Sci* **53**: 1042–1055
- Cathcart JB (1980) World phosphate reserves and resources. In FE Khasawneh, EC Sample, EJ Kamprath, eds, *The Role of Phosphorus in Agriculture*. American Society of Agronomy, Madison, WI, pp 1–18
- Fan MS, Zhu JM, Richards C, Brown KM, Lynch JP (2003) Physiological roles for aerenchyma in phosphorus-stressed roots. *Funct Plant Biol* **30**: 493–506
- Gahoonia TS, Nielsen NE (2004) Root traits as tools for creating phosphorus efficient crop varieties. *Plant Soil* **260**: 47–57
- Gahoonia TS, Nielsen NE, Joshi PA, Jahoor A (2001) A root hairless barley mutant for elucidating genetic of root hairs and phosphorus uptake. *Plant Soil* **235**: 211–219
- Ho MD, Rosas JC, Brown KM, Lynch JP (2005) Root architectural tradeoffs for water and phosphorus acquisition. *Funct Plant Biol* **32**: 737–748
- Hunt R (1982) *Plant Growth Curves: A Functional Analysis Approach to Plant Growth Analysis*. Edwin Arnold, London
- Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ (2006) Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Ann Bot (Lond)* **98**: 693–713
- Liao H, Rubio G, Yan X, Cao A, Brown KM, Lynch JP (2001) Effect of phosphorus availability on basal root shallowness in common bean. *Plant Soil* **232**: 69–79
- Liao H, Yan X, Rubio G, Pedraza F, Beebe S, Lynch JP (2004) Genetic mapping of basal root gravitropism and phosphorus acquisition efficiency in common bean. *Funct Plant Biol* **31**: 1–12
- Lynch JP (2007) Roots of the second green revolution. *Aust J Bot* **55**: 493–512
- Lynch JP (2011) Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. *Plant Physiol* **156**: 1041–1049
- Lynch JP, Brown KM (2001) Topsoil foraging: an architectural adaptation of plants to low phosphorus availability. *Plant Soil* **237**: 225–237
- Lynch JP, Brown KM (2008) Root strategies for phosphorus acquisition. In P White, J Hammond, eds, *The Ecophysiology of Plant-Phosphorus Interaction*. Springer, Dordrecht, The Netherlands, pp 83–116
- Lynch JP, Brown KM (2012) New roots for agriculture: exploiting the root phenome. *Philos Trans R Soc Lond B Biol Sci* **367**: 1598–1604
- Lynch JP, Ho MD (2005) Rhizoeconomics: carbon costs of phosphorus acquisition. *Plant Soil* **269**: 45–56
- Lynch JP, Wojciechowski T (January 11, 2015) Opportunities and challenges in the subsoil: pathways to deeper rooted crops. *J Exp Bot* <http://dx.doi.org/10.1093/jxb/eru508>
- Ma Z, Bielenberg DG, Brown KM, Lynch JP (2001a) Regulation of root hair density by phosphorus availability in *Arabidopsis thaliana*. *Plant Cell Environ* **24**: 459–467
- Ma Z, Walk TC, Marcus A, Lynch JP (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
- Miguel M (2004) Genotypic variation in root hairs and phosphorus efficiency in common bean (*Phaseolus vulgaris* L.). PhD thesis. Pennsylvania State University, University Park
- Miguel MA, Widrig A, Vieira RF, Brown KM, Lynch JP (2013) Basal root whorl number: a modulator of phosphorus acquisition in common bean (*Phaseolus vulgaris*). *Ann Bot (Lond)* **112**: 973–982
- Miller C, Ochoa I, Nielsen KL, Beck D, Lynch JP (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, Riley JP** (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* **27**: 31–36
- Olsen SR, Cole CV, Watanabe FS, Dean LA** (1954) Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. Circular 939. US Department of Agriculture, US Government Printing Office, Washington, DC
- Postma JA, Lynch JP** (2010) Theoretical evidence for the functional benefit of root cortical aerenchyma in soils with low phosphorus availability. *Ann Bot (Lond)* **107**: 829–841
- Postma JA, Lynch JP** (2011) Root cortical aerenchyma enhances the growth of maize on soils with suboptimal availability of nitrogen, phosphorus, and potassium. *Plant Physiol* **156**: 1190–1201
- Rubio G, Walk T, Ge ZY, Yan XL, Liao H, Lynch JP** (2001) Root gravitropism and below-ground competition among neighboring plants: a modeling approach. *Ann Bot (Lond)* **88**: 929–940
- Ryan P, Delhaize E, Jones D** (2001) Function and mechanism of organic anion exudation from plant roots. *Annu Rev Plant Physiol Plant Mol Biol* **52**: 527–560
- Sanchez PA, Uehara G** (1980) Management considerations for acid soils with high phosphorus fixation capacity. *In* FE Khasawneh, EC Sample, EJ Kamprath, eds, *The Role of Phosphorus in Agriculture*. American Society of Agronomy, Madison, WI, pp 471–514
- Serebrovsky AS** (1925) “Somatic segregation” in domestic fowl. *J Genet* **16**: 33–42
- Smith SE, Read DJ** (2008) *Mycorrhizal Symbioses*, Ed 3. Academic Press, New York
- Trachsel S, Kaeppeler SM, Brown KM, Lynch JP** (2011) Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. *Plant Soil* **341**: 75–87
- Vance CP, Uhde-Stone C, Allan DL** (2003) Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytol* **157**: 423–447
- Walk TC, Jaramillo R, Lynch JP** (2006) Architectural tradeoffs between adventitious and basal roots for phosphorus acquisition. *Plant Soil* **279**: 347–366
- Yan XL, Liao H, Beebe SE, Blair MW, Lynch JP** (2004) QTL mapping of root hair and acid exudation traits and their relationship to phosphorus uptake in common bean. *Plant Soil* **265**: 17–29
- York LM, Nord EA, Lynch JP** (2013) Integration of root phenes for soil resource acquisition. *Front Plant Sci* **4**: 355
- Zhu J, Kaeppeler SM, Lynch JP** (2005) Topsoil foraging and phosphorus acquisition efficiency in maize (*Zea mays*). *Funct Plant Biol* **32**: 749–762
- Zhu J, Zhang CC, Lynch JP** (2010) The utility of phenotypic plasticity of root hair length for phosphorus acquisition. *Funct Plant Biol* **37**: 313–322