

Root architectural tradeoffs for water and phosphorus acquisition

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Abstract. Root architectural traits that increase topsoil foraging are advantageous for phosphorus acquisition but may incur tradeoffs for the acquisition of deep soil resources such as water. To examine this relationship, common bean genotypes contrasting for rooting depth were grown in the field and in the greenhouse with phosphorus stress, water stress and combined phosphorus and water stress. In the greenhouse, water and phosphorus availability were vertically stratified to approximate field conditions, with higher phosphorus in the upper layer and more moisture in the bottom layer. Under phosphorus stress, shallow-rooted genotypes grew best, whereas under drought stress, deep-rooted genotypes grew best. In the combined stress treatment, the best genotype in the greenhouse had a dimorphic root system that permitted vigorous rooting throughout the soil profile. In the field, shallow-rooted genotypes surpassed deep-rooted genotypes under combined stress. This may reflect the importance of early vegetative growth in terminal drought environments. Our results support the hypothesis that root architectural tradeoffs exist for multiple resource acquisition, particularly when resources are differentially localised in the soil profile. Architectural plasticity and root dimorphism achieved through complementary growth of distinct root classes may be important means to optimise acquisition of multiple soil resources.

Keywords: drought, functional tradeoffs, multiple resource acquisition, *Phaseolus vulgaris* (common bean), phosphorus, root architecture.

Introduction

Plant adaptation and productivity in a given environment is primarily determined by the ability of an individual or species to acquire resources (Aerts *et al.* 1991; Aerts 1999). Most natural environments are suboptimal with respect to multiple environmental resources (Hutchings *et al.* 2003). Root architecture, the spatial configuration of root systems, is an important factor determining below-ground resource acquisition (Fitter 1988*b*; Lynch 1995; Lynch and Brown 2001). The importance of root architecture for plant productivity stems from the fact that many soil resources are unevenly distributed in space and time and are often subject to localised depletion (Lynch 2005). Thus, spatial deployment of the root system determines the ability of a plant to exploit heterogeneous soil resources (Fitter 1994; Robinson 1996; Lynch and Brown 2001; Hodge 2004). For example, drought tolerance in common bean has been associated with increased rooting depth (Markhart 1985; Sponchiado *et al.* 1989), while greater nutrient acquisition has been associated with topsoil

foraging, especially in the case of immobile resources such as phosphorus (Lynch and Beebe 1995; Bonser *et al.* 1996; Lynch and Brown 2001). Consequently, genetic variation in root architecture among and between species is associated with adaptation and productivity in specific environments (Fitter 1994; Lynch 2005).

Common bean (*Phaseolus vulgaris* L.) is the most important food legume on earth, providing important nutrients for over 500 million people in developing nations (CIAT 2001). Drought and low phosphorus availability are primary constraints to crop production in many developing countries, affecting over 80% of bean production regions in the world, and they frequently co-occur throughout the tropics (CIAT 2001). Bean genotypes vary substantially in their adaptation to low phosphorus availability (Lynch and Beebe 1995) because of differences in acquisition of phosphorus from the soil (Yan *et al.* 1995). Topsoil foraging achieved through root architectural traits is an important adaptation to low phosphorus availability in

Abbreviations used: ANOVA, analysis of variance; DAP, days after planting; RILs, recombinant inbred lines; SRL, specific root length.

this species (Bonser *et al.* 1996; Liao *et al.* 2001, 2004; Lynch and Brown 2001).

Plants typically confront multiple environmental constraints and must co-optimize their resource allocation for acquisition of several limiting resources (Bloom *et al.* 1985; Ho *et al.* 2004). While root architectures that exploit topsoil resources efficiently may be advantageous in low-phosphorus soils, this may inadvertently result in reduced water acquisition, since water availability often increases with soil depth. This is especially true in many rainfed agroecosystems, which experience terminal droughts seasonally, in which the depth of water availability increases through the crop growth cycle. In fact, recent evaluation of phosphorus-efficient germplasm in the tropics suggests that genotypes selected for adaptation to low-phosphorus soils may be sensitive to drought (S Beebe pers. comm.). Thus, there may be a trade-off in the optimization of root architecture for shallow immobile resources such as phosphorus and deep mobile resources such as water.

Although we are beginning to understand some of the architectural adaptations of root systems to individual soil constraints, little is known about how such traits affect adaptation to multiple soil constraints. In this study, we focus on the circumstance of suboptimal water and phosphorus availability, two principal soil constraints to plant growth in terrestrial ecosystems. Water and phosphorus are also interesting since they represent extremes of contrasting resource availability in time and space, where water is ephemeral, mobile and usually deep, whereas phosphorus is stable, immobile and usually shallow. Architectural strategies that optimize phosphorus acquisition would also be beneficial for the acquisition of other immobile resources such as the micronutrient metals, whereas traits optimizing water acquisition would also assist in the acquisition of soluble mobile resources such as nitrate. While numerous studies have focused on the interaction of water and phosphorus acquisition for plant growth (e.g. Fitter 1988a; Al-Karaki *et al.* 1995), we are not aware of any studies describing specific root traits that enable a plant to co-optimize acquisition of both of these resources. The primary objective of this work was to understand the tradeoffs of root architectural strategies for water and phosphorus acquisition. Our specific hypotheses were: (1) shallow-rooted genotypes will perform better in environments where phosphorus availability is localised in surface soil horizons, (2) deep-rooted genotypes will perform better in terminal drought environments and (3) genotypes that are adapted to both water and phosphorus limited environments will have dimorphic root characteristics.

Materials and methods

Experiments were conducted in two locations, a controlled environment greenhouse at University Park, PA, USA and a field site at Zamorano, Honduras.

Stratified water and phosphorus greenhouse system

Plant material

Seeds of common bean (*Phaseolus vulgaris* L.) genotypes DOR364, G19833 and BAT477 were obtained from the International Center for Tropical Agriculture (CIAT, Cali, Colombia). DOR364, a Mesoamerican genotype with an indeterminate erect bush growth habit, has a deep root architecture when grown in a pouch system, in sand culture and in the field (Bonser *et al.* 1996; Liao *et al.* 2001) and has been characterised as phosphorus-inefficient, but responsive to phosphorus fertilisation (CIAT 1987). G19833, an Andean genotype with an indeterminate prostrate growth habit, has a shallow root system (Bonser *et al.* 1996; Liao *et al.* 2001) and has been characterised as phosphorus-efficient based on plant growth and seed yield in relation to phosphorus availability (Liao *et al.* 2001, 2004). BAT477, a Mesoamerican genotype with an indeterminate erect bush growth habit, has shallow basal roots (Bonser *et al.* 1996; Liao *et al.* 2001), but is also tolerant to drought due to a high rooting density at depth (Sponchiado *et al.* 1989).

Growth conditions

Seeds of the three genotypes were surface-sterilised in 10% NaOCl for 5 min, rinsed thoroughly with distilled water and scarified with a sharp razor blade. Seedlings were germinated in rolls of brown germination paper (Anchor Paper Co., St. Paul, MN) soaked in 0.5 mM CaSO₄ and placed in a dark growth chamber for 48 h at 28°C. The seedlings were then planted at a depth of 4 cm, one per pot. Plants were grown in 25.5-L containers (30 cm diameter × 36 cm height) filled with a mixture (2 : 4 : 4 v/v) of yellow sand, coarse grade vermiculite and coarse grade perlite. Each container was stratified into two layers. The layers were separated by a 2-mm fibreglass mesh embedded with a paraffin-coconut oil mixture (3 : 7 v/v), which was placed at 8 cm depth from the surface. One of four treatment combinations of water and phosphorus, (1) control (high water and high phosphorus in both layers), (2) stratified low phosphorus (high phosphorus in the top 0–8 cm and low phosphorus in the bottom 8–36 cm, high water in both layers), (3) stratified low water (low water in the top 0–8 cm and high water in the bottom 8–36 cm and high phosphorus in both layers) and (4) stratified low water and phosphorus (high phosphorus and low water in the top 0–8 cm and low phosphorus and high water in 8–36 cm horizon), was imposed as shown in Fig. 1. Phosphorus levels in each horizon were maintained by incorporating solid-phase-buffered Al-P (1.5% w/v), which maintained phosphorus at either low (5 µM) or high (250 µM) concentration in the soil solution (Lynch *et al.* 1990). Moisture levels in the top and bottom pot layers were maintained by differentially irrigating each horizon. A separate irrigation ring was placed below the mesh in each pot, which was watered twice daily (0800 h and 1500 h), while the top horizon was watered once a day (0800 h) with ~250–350 mL of nutrient solution. Preliminary experiments showed that this was a sufficient volume of moisture to keep the water content of the top layer above 22%, without causing waterlogging. Water from the top horizon was withheld from the water stress treatments 7 d after planting (DAP). The top horizon was always watered first, followed by irrigation of the bottom horizon to saturation. Preliminary studies with the paraffin-coconut oil mesh horizon showed that it did not inhibit root growth or architecture of common bean and was water repellent, in that it minimised moisture movement between soil horizons. The nutrient solution used in the irrigation regime contained (in mM): 3.1 NO₃, 1.8 K, 1.2 Ca, 1.4 SO₄, 1.0 NH₄, 0.825 Mg, 0.05 Cl, and (in µM) 5 Fe-EDTA, 2 B, 1.5 Mn, 1.5 Zn, 0.143 Mo and 0.5 Cu. All plants were grown between April and June 2002 in a controlled environment greenhouse at University Park, PA (40°85'N, 77°83'W). The temperature ranged from a maximum of 28°C (day) to a minimum of 22°C (night). The relative humidity was kept at 50–60% in the daytime and 60% at night. Ambient light, which ranged from

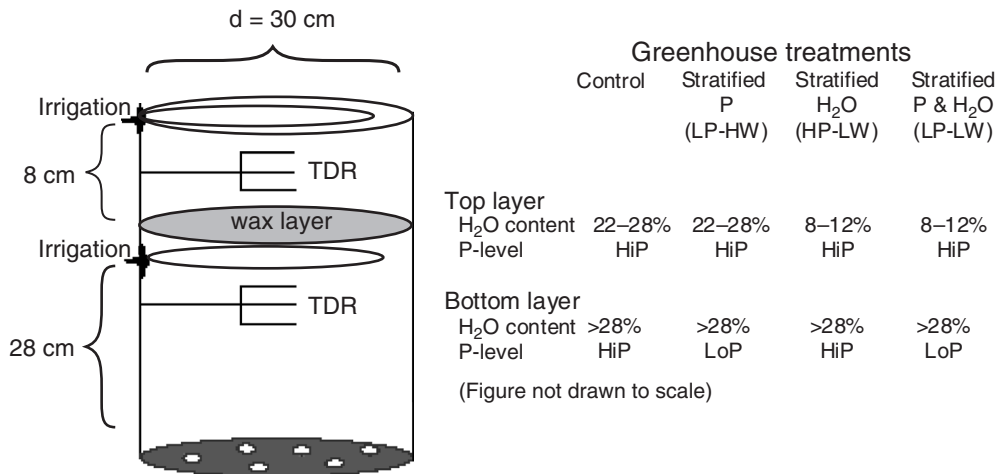


Fig. 1. Stratified water and phosphorus greenhouse system. Shallow (0–8 cm) and deep (8–35 cm) layers are separated by a paraffin–coconut oil mesh layer and irrigated independently. The water and phosphorus levels of each layer are given for each treatment. Phosphorus concentration in each layer was maintained by AI-P buffered media at either high (250 μM) or low (5 μM) phosphorus. Water content of each layer was monitored by TDR probes.

400 to 1800 μmol at midday, was supplemented from 0800 to 1800 h with 110 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ from 400-W metal-halide bulbs (Energy Technics, York, PA) when ambient light levels were below 400 μmol . The plants were harvested 32–33 DAP, during the late vegetative stage of development.

Leachate samples were collected from each pot and analysed for phosphorus concentration (Murphy and Riley 1962) twice during the experiment (7 and 21 DAP). Moisture content was monitored in the top horizon of all containers with a TDR-100 multiplexed time-domain reflectometry system (Campbell Scientific Inc., Logan, UT). Measurements of each TDR probe were made every half hour and data were logged and stored by a CR21X datalogger (Campbell Scientific Inc.). Preliminary experiments showed that the irrigation regime imposed was sufficient to ensure that the bottom horizon would stay at a moisture content well above 28%, so TDR probes were only used to monitor the top horizon in every pot throughout the duration of the experiment. The day before harvest, pre-dawn leaf water potential and leaf osmotic potential were determined with a Scholander pressure chamber (Soil Moisture Inc., Santa Barbara, CA) and a vapour pressure osmometer (Wescor Scientific, Logan, UT), respectively. Leaf gas-exchange measurements were also made at 21 and 28 DAP with a portable open system infrared gas-exchange system (LiCor 6400, Li-Cor, Lincoln, NE).

Experimental design

Each genotype was grown at each of the four treatment combination levels. Five replicates were planted and measured 2 d apart. Plant and treatment positions were arranged in a randomised complete block design with time of planting as the block.

Biomass, phosphorus and root measurements

Plants were harvested for shoot and root biomass 32–33 DAP. Shoot tissue was dried at 60°C for 3–5 d and weighed. Subsamples of leaf biomass were ground, ashed at 500°C for 10 h and analysed for phosphorus content spectrophotometrically (Murphy and Riley 1962). Roots were harvested by root type and by horizon. A single basal root from both the top and bottom layers was subsampled for root length analysis, while the entire tap root from both layers was scanned and

analysed for root length. All root samples were kept in ethanol (25% v/v) prior to root length analysis. Root samples were rinsed to remove the ethanol and dyed in neutral red (0.16 g L⁻¹, Sigma Chemical, St Louis, MO) for at least 24 h before scanning. Root images were scanned and analysed with the WinRhizo Ver 4.1 PRO imaging system (Regent Instruments, Quebec, Canada) for total root length and total surface area, as well as root length and root area by root diameter class. After scanning, root samples were dried at 60°C for 48 h and weighed in order to determine specific root length (mg^{-1}) of each sample. Relative shoot biomass was determined to be the fraction of shoot biomass relative to the total biomass (g g^{-1}).

Water and phosphorus field experiment — Zamorano, Honduras

Plant material — L88 recombinant inbred population

Upon completion of the greenhouse work, we discovered the existence of a set of recombinant inbred lines (RILs) that were segregating for tolerance to drought and low phosphorus availability. RILs are powerful tools because they segregate for various traits but share the same genetic background and are useful for investigating the tradeoffs of contrasting root architecture. We hypothesised that root system parameters, specifically rooting depth, would co-segregate with tolerance to the different environmental stress.

Two small-seeded black bean genotypes, B98311 and TLP19, were developed by Dr J Kelly at Michigan State University and crossed to generate a recombinant inbred (RI) population, the L88 population consisting of 81 lines (Frahm *et al.* 2004). B98311 is a drought-resistant Mesoamerican genotype from the Michigan State University bean breeding program that possesses a Type II growth habit and a deep vigorous taproot (Frahm *et al.* 2004). TLP19 was developed for tolerance to low phosphorus at CIAT and it possesses a Type II growth habit and is also of the Mesoamerican gene pool. This population has been screened for performance under drought conditions at three field locations (Frahm *et al.* 2004) and was also screened for basal root shallowness in high and low phosphorus conditions (Ho *et al.* 2004). On the basis of the screening results, four RILs, two with shallow basal roots (L88–43, L88–57) and two with deep basal roots (L88–30, L88–63) were selected for this study, in addition to the two parent genotypes.

Growth conditions

A field experiment was conducted from late January to early May 2004 during the dry summer season at the Escuela Agrícola Panamericana (Zamorano), Honduras (14°6'N, 87°13'W). The study consisted of six common bean genotypes, three shallow and three deep genotypes, grown in high and low phosphorus conditions with and without irrigation. A 25 m × 110 m (2750 m²) field (Mollisol) was used for this study. The south half (25 m × 55 m) of the field had low soil phosphorus availability (<3–5 ppm, Olsens method). The entire field was divided in half east–west, where the eastern half of the field received irrigation throughout the entire trial while the western half did not. The treatments were imposed as follows: (1) HP-IR, high phosphorus, with irrigation (northeast quadrant), (2) LP-IR, low phosphorus, with irrigation (southeast quadrant), (3) HP-NI, high phosphorus, with no irrigation (northwest quadrant), and (4) LP-NI, low phosphorus, with no irrigation (southwest quadrant). There were buffer rows surrounding the entire field. In addition, there was a 5-m buffer zone separating the irrigated and non-irrigated portions of the field, and an 8-m buffer zone separating the high phosphorus and low phosphorus fields. Furrow spacing was 0.7 m and the within-row plant spacing was 8 cm. The field was planted in a split-split-plot design, where phosphorus and water levels were each main factors. Each of the four main plots was blocked into five replicates, where eight 2.8 m × 5 m (14 m²) plots were included per block. The six genotypes were randomly assigned to a plot within each block. The entire field was cultivated and planted by hand according to local practices for common bean. The entire field received 40 kg N ha⁻¹ as urea. In addition, the high phosphorus field received 70 kg P ha⁻¹ as triple superphosphate. The entire field was sprinkler irrigated within 2–3 DAP to ensure uniform germination and then every 7–10 d. Irrigation to the dry side was terminated at 21 DAP. The irrigated half of the field received furrow irrigation every 7–10 d for the duration of the growing season. Field water content was monitored at three depths (0–30 cm, 30–60 cm, >60 cm) every 7–10 d up to the time of pod set with the TRIME system (Mesa Systems, Medfield, MA). Average water content reached 5–10%, 10–15% and 18–25% for the 0–30 cm, 30–60 cm and 60–90 cm in the unirrigated treatment, while they were 15–20%, 20–28% and 25–35%, in the irrigated side.

Biomass, phosphorus and root length density measurements in the field

Biomass samples were harvested at 21, 35 and 44 DAP. Three plants of each genotype were harvested, dried at 60°C and weighed. Dried shoot samples were analysed for phosphorus as above. Soil cores were collected manually at 39–41 DAP with a 5-cm diameter soil coring apparatus (Giddings Machine Co, Windsor, CO). Cores were taken to a 60-cm depth and separated into three layers, 0–20 cm, 20–40 cm and 40–60 cm and washed to remove soil. Root samples were collected, stored in 25% (v/v) alcohol and transported to University Park, PA, where they were scanned and analysed for root length as described above.

Statistical analyses

Data from the greenhouse study were analysed as a randomised-complete block design with the MiniTab statistical software Ver. 14 (MiniTab Inc., State College, PA). For the greenhouse experiment, a fixed effect model analysis of variance (ANOVA) was conducted for shoot biomass, root biomass, root:shoot ratio, relative root biomass in the 0–8 cm horizon, where genotype, phosphorus and water treatments, and replicate were the independent variables. A fixed effect model ANOVA was also conducted for total root biomass, total and relative tap root biomass and specific root length, where genotype, phosphorus and water treatments, root type, pot depth and replicate were the independent variables for the greenhouse experiment.

Data from the field study were analysed as a split-split-plot design with MiniTab. The three shallow genotypes and the three deep genotypes were grouped together *a priori* into a shallow and deep rooting class, respectively, based upon a previous screening study (Ho 2004). A random model nested analysis of variance was conducted for shoot biomass for a given water and phosphorus level in the field, where class is the main factor and genotype was nested. A fixed model nested analysis of variance was conducted for root length density for a given water and phosphorus level in the field, where class and soil depth were the main factors and genotype was nested.

All data were tested for normality before carrying out ANOVA. Log transformation of data before ANOVA was employed as needed, when variance was found to be correlated with means.

Results

Plant performance in a stratified water and phosphorus greenhouse system

Overall plant performance in the stratified water and phosphorus system in the greenhouse was assessed by vegetative biomass accumulation and phosphorus acquisition. There was no difference between genotypes in biomass production under unstressed conditions (Fig. 2a). BAT477 performed equally or better than the other genotypes

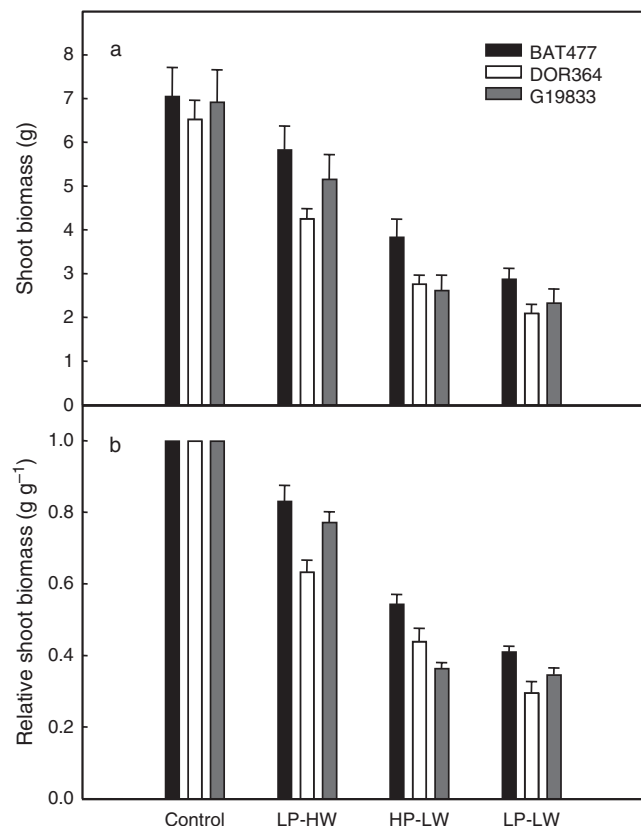


Fig. 2. Plant performance as given by shoot biomass (a) and relative shoot biomass as a percentage of control (b) for three common bean genotypes in stratified sand control. 'LP' = low phosphorus, 'HP' = high phosphorus, 'LW' = low water, 'HW' = high water availability. Within a panel, bars under a different letter are statistically different at $P < 0.05$. Bars indicate standard error. $n = 4$.

in all low water and phosphorus treatment combinations ($P < 0.05$; Fig. 2). BAT477 performed better in terms of absolute (Fig. 2a) or relative plant performance. The performance of the shallow-rooted genotype G19833 was better than that of the deep-rooted genotype DOR364 in the stratified low phosphorus treatment ($P \leq 0.05$; Fig. 2b), while DOR364 outperformed G19833 in the stratified low water treatment ($P \leq 0.01$; Fig. 2b). There was no statistical difference between the performance of G19833 and DOR364 in the combined low phosphorus and low water treatment. Similar genotype rankings were obtained with total plant phosphorus content (Fig. 3). There was no difference between genotypes in phosphorus concentration, but there was a significant effect of stress treatments, all of which decreased tissue phosphorus concentration ($P \leq 0.001$; Fig. 3a).

Root allocation and localisation differs between genotypes

There was a significant effect of genotype and water treatment on total root biomass, although root:shoot ratio was

significantly affected only by genotype and the interaction of the water and phosphorus treatments ($P \leq 0.001$; Fig. 4). BAT477 produced more total root biomass, regardless of treatment, compared with the other two genotypes (Fig. 4a). The difference was most pronounced in the stratified phosphorus treatment, which resulted in higher total root biomass than the unstressed control. The three genotypes differed in root biomass localisation. BAT477 and G19833 localised more root biomass than DOR364 in the 0–8 cm horizon, particularly when phosphorus was limiting (Fig. 5a). BAT477 allocated a large proportion of root biomass to the deep horizon, especially when water was limiting (Fig. 5b). This resulted in a smaller relative shallow root fraction for BAT477 and a larger shallow root fraction for G19833 (Fig. 5c). DOR364 had intermediate rooting depth. The relative shallow root fraction was not correlated with either shoot or root size for any genotype (Fig. 6) and therefore, reflects a difference in allocation strategies between genotypes rather than a byproduct of allometry. Indeed, there were no differences among genotypes in the allometric coefficient for root–shoot partitioning, when all treatments were grouped together (data not shown).

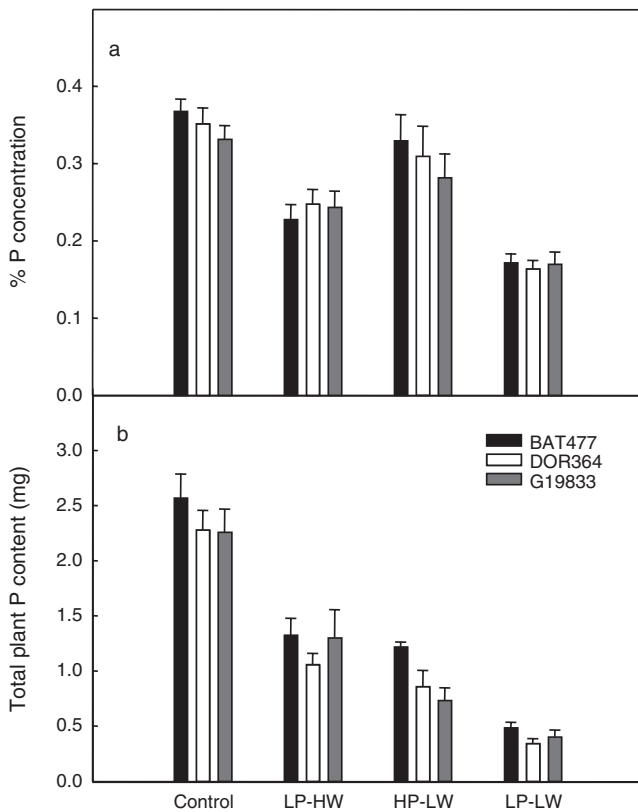


Fig. 3. Tissue phosphorus concentration (a) and total phosphorus content (b) for three common bean genotypes in stratified sand culture. ‘LP’ = low phosphorus, ‘HP’ = high phosphorus, ‘LW’ = low water, ‘HW’ = high water availability. Within a panel, bars under a different letter are statistically different at $P < 0.05$. Bars indicate standard error. $n = 4$.

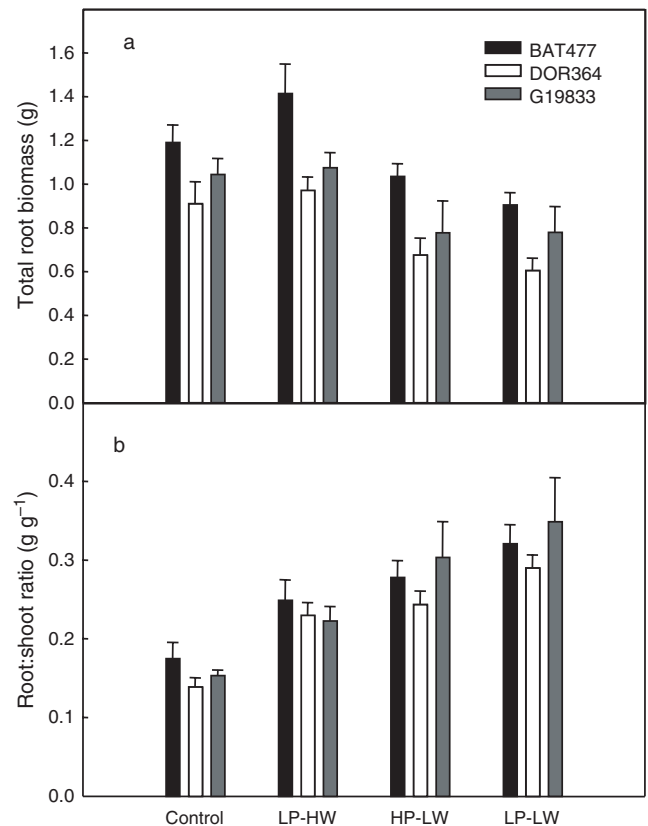


Fig. 4. Total root biomass (a) and root:shoot ratio (b) of three bean genotypes in stratified sand culture. ‘LP’ = low phosphorus, ‘HP’ = high phosphorus, ‘LW’ = low water, ‘HW’ = high water availability. Within a panel, bars under a different letter are statistically different at $P < 0.05$. Bars indicate standard error. $n = 4$.

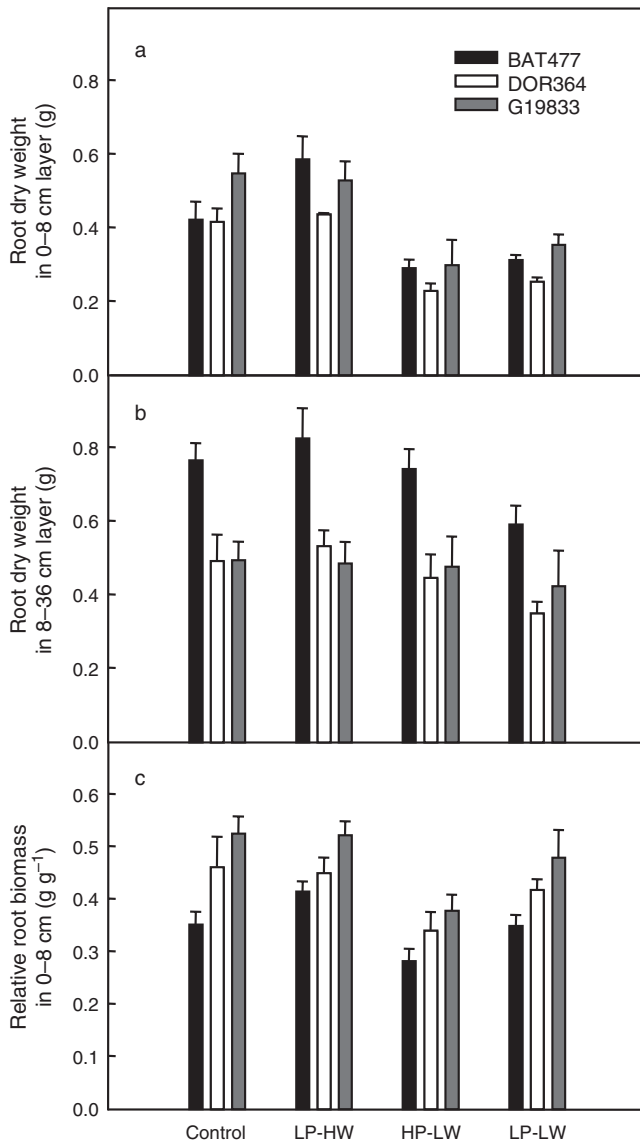


Fig. 5. Root biomass allocation by depth in three bean genotypes in stratified sand culture. ‘LP’ = low phosphorus, ‘HP’ = high phosphorus, ‘LW’ = low water, ‘HW’ = high water availability. Bars indicate standard error. $n = 4$.

In addition to differences in root localisation, there were also differences among genotypes for allocation to different root types (Fig. 7). In general, BAT477 allocated more biomass to the tap root (>20%), especially in the deeper horizon (>30%) in all environments (Fig. 7). G19833 allocated less biomass to the tap root (<20%) and more to basal roots in both the shallow and deep horizon in all environments (Fig. 7). DOR364 increased its allocation to the tap root when water was limiting, particularly in the deep horizon (Fig. 7). There was a significant effect of root type ($P \leq 0.001$) and phosphorus ($P \leq 0.10$)

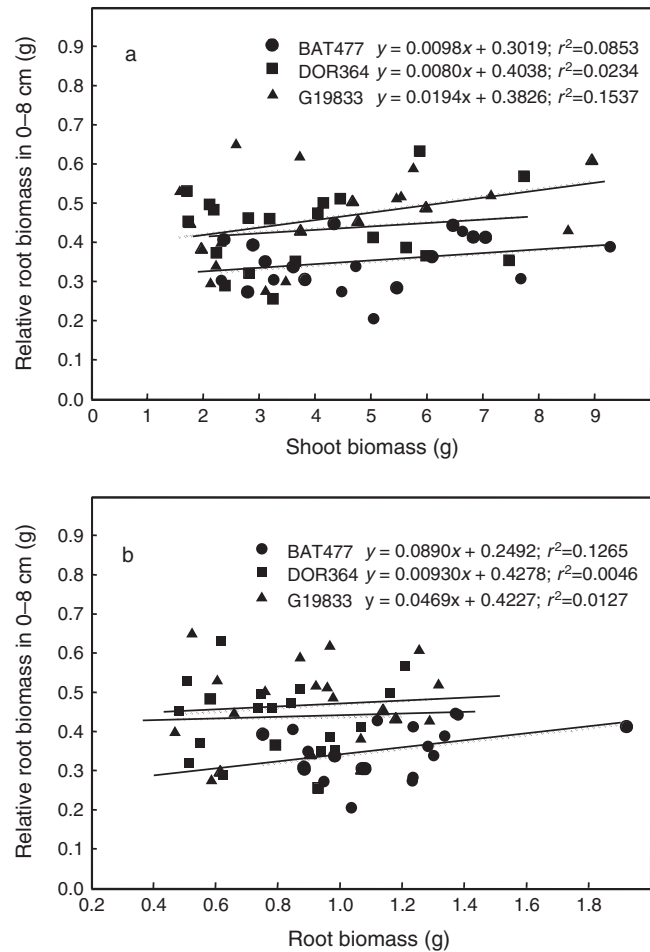


Fig. 6. The relationship of relative root biomass fraction in the shallow 0–8 cm layer to shoot biomass (a) and total root biomass (b) of three bean genotypes growing in stratified sand culture.

on specific root length [SRL, root length per unit dry weight ($m\ g^{-1}$)], which may have important implications for resource acquisition efficiency. Basal roots (including laterals) in general had greater SRL than tap roots in the 0–8 cm horizon (Fig. 8). In general, there were no genotypic differences for SRL, except for the basal and tap root SRL of G19833, which had significantly greater SRL in phosphorus limiting environments (Fig. 8). The tap root of DOR364 had significantly higher SRL in the 8–36 cm layer, compared with the other two genotypes. The basal root of DOR364 was also significantly greater in the 8–36 cm layer relative to the other genotypes for the water stress treatments (Fig. 8).

Field results with recombinant inbred lines

No significant differences were found between shallow and deep genotype classes for biomass production in the field under high phosphorus, irrigated conditions (Fig. 9). The shallow genotype class performed better in the low

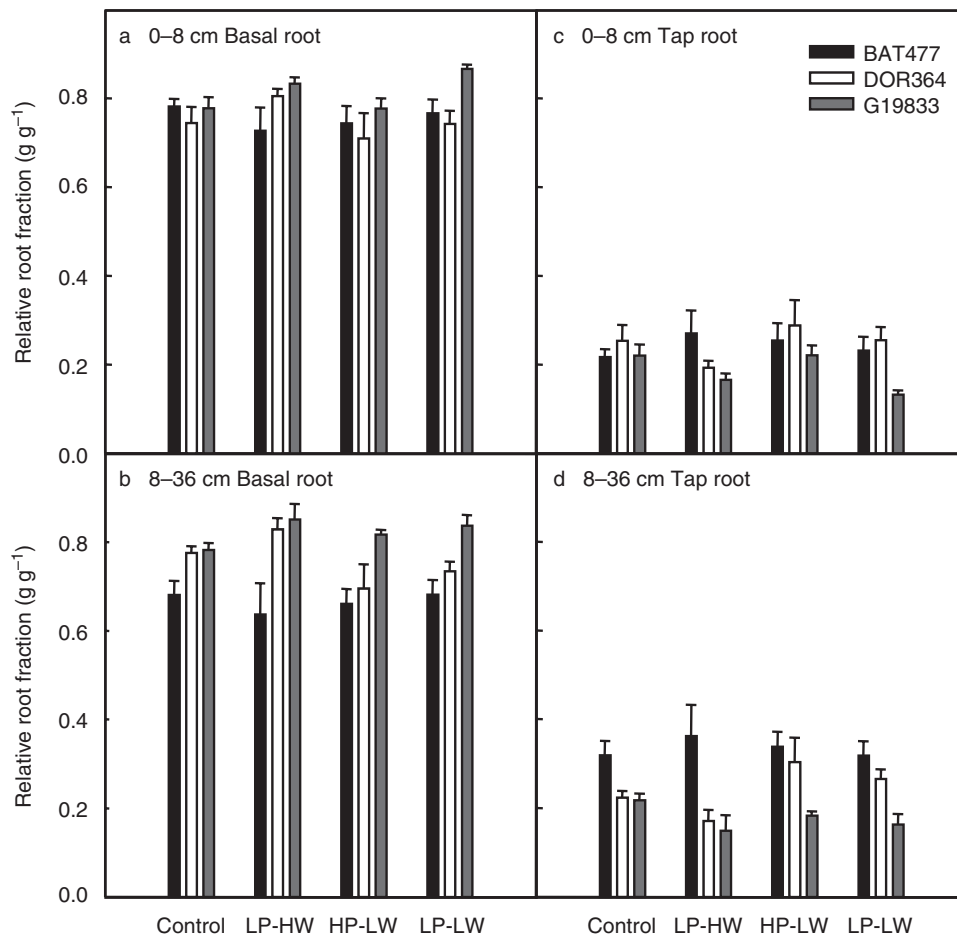


Fig. 7. Relative biomass allocation to basal roots (*a*, *b*) and taproots (*c*, *d*) by soil depth for three bean genotypes in a stratified sand culture. ‘LP’ = low phosphorus, ‘HP’ = high phosphorus, ‘LW’ = low water, ‘HW’ = high water availability. Bars indicate standard error. $n = 4$.

phosphorus irrigated treatment ($P \leq 0.01$; Fig. 9). The deep genotype class performed better in the high phosphorus unirrigated treatment ($P \leq 0.05$; Fig. 9). In the combined water and phosphorus stress treatment, the shallow genotypes performed better ($P \leq 0.05$; Fig. 9). The shallow genotypes had significantly greater total plant phosphorus content in all of the stress treatments in the field ($P \leq 0.001$; Fig. 10), although there was no difference between genotype classes in the unstressed treatment. The shallow genotype class had significantly greater total root length density in the low water and phosphorus treatments ($P \leq 0.001$; Fig. 11). In addition, the shallow genotype class had a larger proportion of root length in shallow soil layers ($P \leq 0.001$; Fig. 11).

Discussion

The overall objective of this work was to assess the physiological tradeoffs for root architectural phenotypes in heterogeneous environments. Specifically, we hypothesised that whereas shallow-rooted genotypes would be better

adapted to phosphorus stress, deep-rooted genotypes would be better adapted to terminal drought. Our results from the greenhouse and field with parent and recombinant inbred lines of common bean support this hypothesis (Figs 2, 9). These results were obtained with both parent genotypes in the greenhouse and a different set of parent and RIL genotypes in the field. While the field is more realistic, it also includes many potentially confounding environmental factors. The fact that results from a controlled environment were confirmed in the field validates our greenhouse methods and also indicates that the architectural traits we examined are robust across environments. The agreement of results from parental genotypes and RILs offers strong support for our hypotheses, since RILs differ in root architecture but share a common genetic background, thereby minimising the potentially confounding effects of other plant traits on stress adaptation and resource acquisition, while comparison of parents reduces the potentially confounding effects of genetic linkage within a single set of RILs.

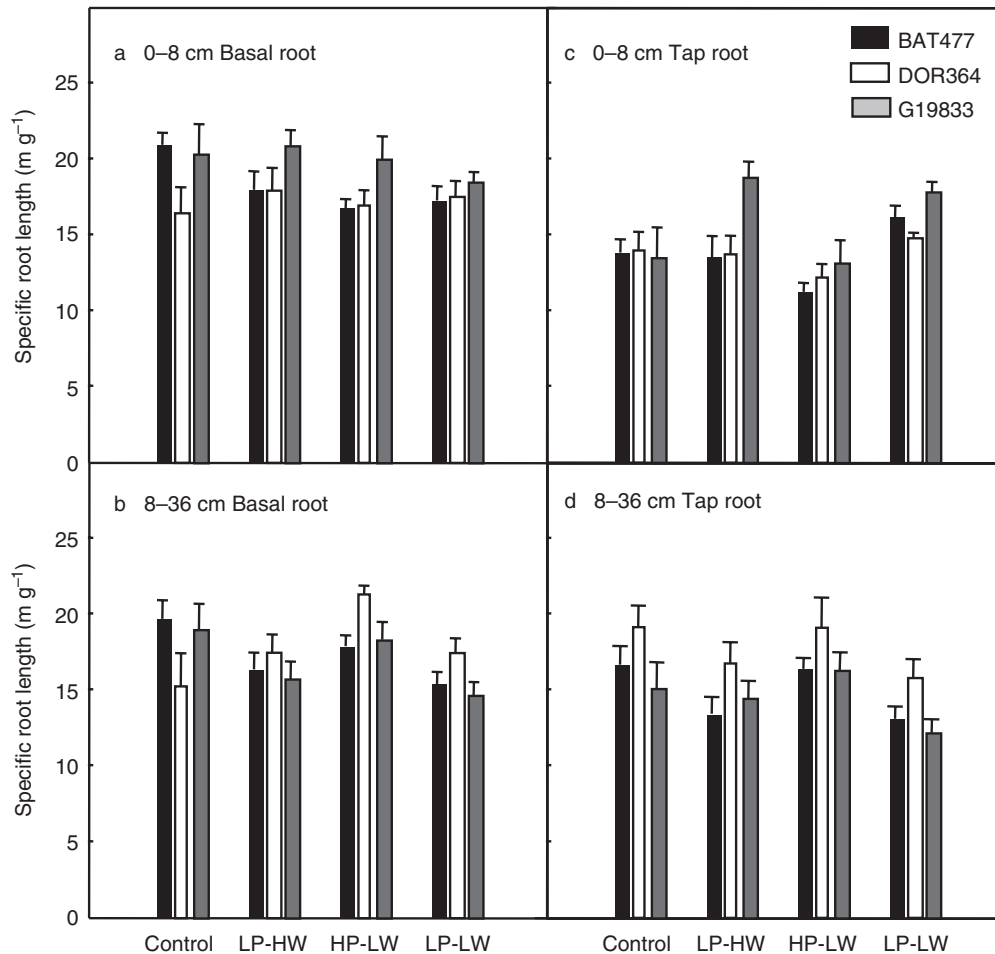


Fig. 8. Specific root length for basal roots (*a, b*) and taproots (*c, d*) by soil depth for three bean genotypes in a stratified sand culture. 'LP' = low phosphorus, 'HP' = high phosphorus, 'LW' = low water, 'HW' = high water availability. Bars indicate standard error. $n = 4$.

Root system size is important for resource acquisition

Root size is important for increased resource acquisition when resources are limiting. BAT477, which performed the best in all treatments, produced more total root biomass in absolute terms than either G19833 or DOR364 (Fig. 4*a*). Having a greater total root biomass allowed BAT477 to have root biomass in the surface horizon that was comparable to the shallow-rooted genotype G19833, especially in the stratified low phosphorus treatment (Fig. 5*a*), while maintaining substantially more biomass in the deep horizon than the other genotypes (Fig. 5*b*). This resulted in a relatively smaller fraction of root biomass in the surface horizon in BAT477, (Fig. 5*c*) even though there was comparable root biomass in the surface layers. Although BAT477 had greater root production in absolute terms, it did not have a greater relative allocation to roots compared to the other two genotypes (Fig. 4*b*). In general, many studies have found an increase

in root : shoot ratio with decreasing soil resource availability [e.g. phosphorus stress in bean (Nielsen *et al.* 2001) and water stress in bean (Sponchiado *et al.* 1989)]. An increased root : shoot ratio is presumably advantageous because it enhances soil resource acquisition, but it does so at the expense of photosynthetic carbon gain. As a result, increased allocation to roots has a carbon cost that can ultimately result in reduced plant growth (Nielsen *et al.* 2001). In this study, all three genotypes had comparable increases in the root : shoot ratio in response to stress treatments. There were also no significant differences among genotypes in the root : shoot allometric coefficient when all treatments were considered together (data not shown). BAT477 maintains a larger root system, but not at the expense of biomass allocation to shoots. Thus, while root size is likely to be important for resource acquisition, other architectural traits are likely to also play a role, such as spatial localisation and carbon allocation to specific root types.

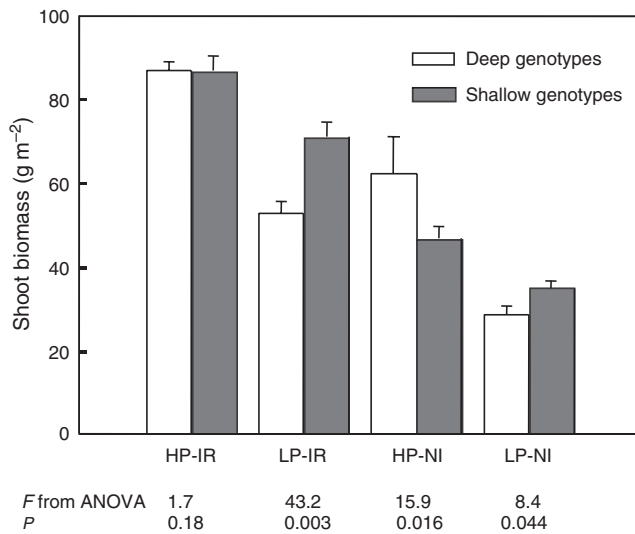


Fig. 9. Shoot biomass at 44 DAP for three shallow-rooted and three deep-rooted common bean genotypes in the field. ‘HP’ = high phosphorus availability, ‘LP’ = low phosphorus availability, ‘IR’ = irrigated, ‘NI’ = non-irrigated. *F* and *P* values from ANOVA test the significance of the difference between deep- and shallow-rooted genotypes within any given treatment.

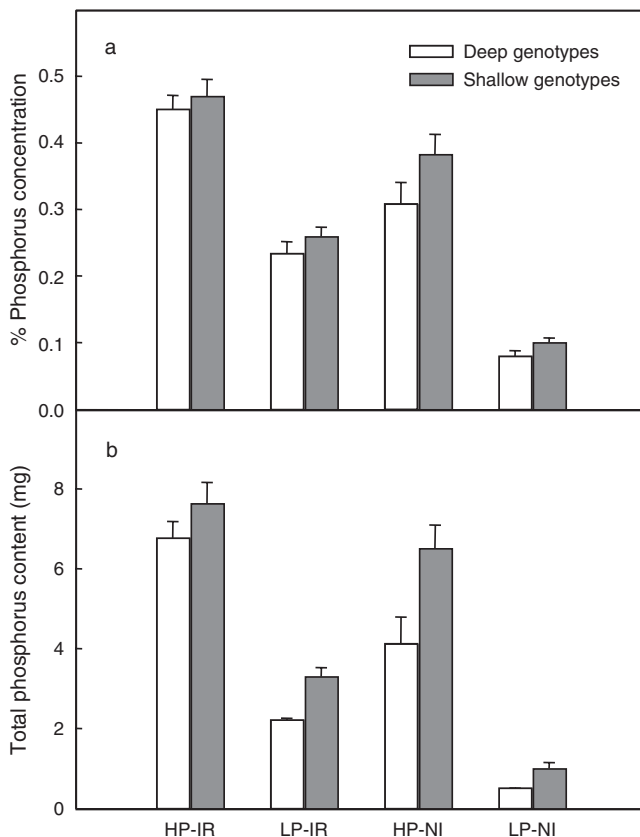


Fig. 10. Phosphorus concentration (a) and total phosphorus content (b) three shallow-rooted and three deep-rooted common bean genotypes in the field. ‘HP’ = high phosphorus availability, ‘LP’ = low phosphorus availability, ‘IR’ = irrigated, ‘NI’ = non-irrigated.

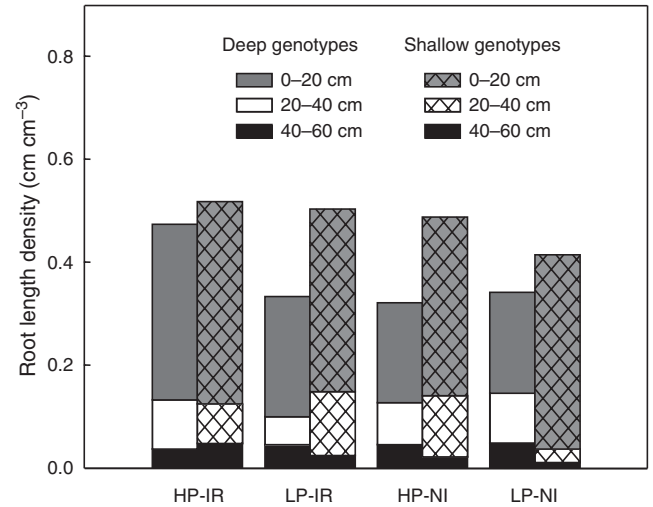


Fig. 11. Root length density of shallow- and deep-rooted genotypes of common bean at three soil depths in the field. ‘HP’ = high phosphorus availability, ‘LP’ = low phosphorus availability, ‘IR’ = irrigated, ‘NI’ = non-irrigated.

Spatial deployment of root biomass affects acquisition of water and phosphorus

Our results demonstrate the benefits from co-localising root growth and resource availability in spatially heterogeneous environments. The shallow-rooted genotype, G19833, had a greater relative root fraction in the shallow horizon (Fig. 5c) and as a result outperformed the deep-rooted genotype in the stratified low phosphorus treatment (Figs 2, 3). Similarly, in the field, shallow-rooted genotypes, which had greater root length density in the surface horizons relative to deep-rooted genotypes (Fig. 11), had greater biomass (Fig. 9) and greater total phosphorus accumulation (Fig. 10) under phosphorus stress. Conversely, the deep-rooted genotypes performed better in the terminal drought environment when phosphorus was sufficient. Optimisation modelling indicates that the optimal rooting depth for multiple resource acquisition will ultimately depend on the relative scarcity and localisation of one resource compared with the other (Ho *et al.* 2004).

Spatial localisation of roots is an important root architecture strategy for efficient resource acquisition when resources are heterogeneous in time and space (Drew and Sacker 1978; Sharp and Davies 1985; Fitter *et al.* 1991; Snapp *et al.* 1995; Leyser and Fitter 1998; Leffler *et al.* 2004). Topsoil foraging is an important component of acquisition of nutrients such as phosphorus, which are immobile and localised at the soil surface (Lynch and Brown 2001). Several root architectural traits contribute to overall root system shallowness, including basal root growth angle (Bonser *et al.* 1996) and adventitious root production (Miller *et al.* 2003). Patch proliferation of roots can also be an effective means of increasing nutrient acquisition efficiency and can

affect competition and community dynamics (Pregitzer *et al.* 1993; Larigauderie and Richards 1994; Snapp *et al.* 1995; Robinson 1996; Leyser and Fitter 1998). Root system plasticity has long been associated with increased nutrient acquisition and adaptation in heterogeneous environments (Jackson *et al.* 1990; Fitter 1994; Hutchings and de Kroon 1994) and is thus, considered to be an important aspect of root foraging (Hutchings and de Kroon 1994; de Kroon and Hutchings 1995; Lynch and Brown 2001), although tradeoffs to root plasticity also exist (Ho *et al.* 2004). In addition to resource acquisition, inter- and intra-specific segregation of root systems may also be important in reducing competition and facilitating species coexistence and niche differentiation (Berendse 1981, 1983; Hutchings and John 2003; de Kroon *et al.* 2003).

Shallow-rooted genotypes performed slightly better than deep-rooted genotypes in the combined stress treatment in the greenhouse (not significant) and in the field ($P < 0.05$). We interpret this as evidence that shallow roots permit greater phosphorus acquisition and therefore, promote early seedling growth, which enhances water acquisition in gradually drying soil (Al-Karaki *et al.* 1995). In effect, having shallow roots and acquiring phosphorus early is essential for adequate growth and survival of a drought later in the season.

Allocation to specific root types affects whole-root-system resource acquisition efficiency

In addition to root localisation, carbon allocation to specific root types may be an important strategy for resource acquisition efficiency. G19833 consistently allocated a larger fraction of root biomass to basal roots than to tap roots, regardless of horizon depth and treatment (Fig. 7). In contrast, BAT477 consistently allocated a greater fraction of root biomass to the tap root, regardless of horizon and treatment (Fig. 7). DOR364 allocated a greater fraction to basal roots when phosphorus was limiting and less to basal roots when water was limiting (Fig. 7). Basal roots may be more important than tap roots for phosphorus acquisition because they have greater SRL (Fig. 8). The phosphorus-efficient G19833 had especially high basal root SRL (Fig. 8). Changes in SRL and root length density in response to resource availability can be adaptive (Larigauderie and Richards 1994; Rodrigues *et al.* 1995; Fransen *et al.* 1998). The tap root for all genotypes in the greenhouse study had consistently lower SRL than basal roots (Fig. 8). This difference in SRL for tap roots was much less pronounced in the bottom horizon, particularly for DOR364. Because water is more mobile than phosphorus, root absorptive surface area and length is not as critical for water acquisition as it is for phosphorus acquisition. In fact, larger diameter roots may have greater hydraulic conductance, which would facilitate more efficient water transport (Passioura 1988; Doussan *et al.* 1998).

Plants can increase their nutrient-acquisition efficiency through cost minimisation in low-phosphorus soils (Lynch and Ho 2005). Different root types differ in their metabolic costs to the plant. Under low-phosphorus conditions, phosphorus-efficient genotypes shift root biomass allocation to favour more metabolically efficient root classes, such as adventitious and basal roots (Miller *et al.* 2003), although adventitious roots were not specifically addressed in the present study. In beans, adventitious and basal roots have greater SRL and lower linear construction cost (grams of carbon or glucose equivalents per unit root length, g GE cm^{-1}) than tap roots (Miller *et al.* 2003). In addition to shifting biomass to adventitious and basal roots, phosphorus-efficient genotypes have reduced specific respiration at low phosphorus for a given root class (MD Ho and JP Lynch unpubl. data), which in this case could be due to anatomical adaptations to low phosphorus availability that reduce root costs. Although aerenchyma formation is commonly regarded as an adaptation to hypoxia (Drew *et al.* 2000), it is also induced by low nitrogen or phosphorus availability in well-aerated roots of maize (Drew *et al.* 1989). This response is also observed in rice (Lu *et al.* 1999) and common bean (Fan *et al.* 2003). In maize and bean, genotypes with superior adaptation to low-phosphorus soil had greater aerenchyma formation, which proportionately reduced root tissue phosphorus content and disproportionately reduced root respiration (Fan *et al.* 2003). Thus, allocation to specific root classes can have significant effects on the overall acquisition efficiency of a root system.

Importance of a dimorphic root system for multiple resource acquisition

The success of BAT477 in all stress treatments suggests that a root architecture that allows for both shallow and deep root localisation is best for adaptation to multiple resource limitations. Dimorphism or polymorphism within the root system and architectural plasticity in response to resource availability could be important in co-optimising the acquisition of multiple soil resources. BAT477 maintains comparable biomass in the soil surface relative to the shallow genotype, G19833. This shallow root fraction is comprised to a large extent of basal roots, which have a greater SRL relative to the tap root, which is important for efficient acquisition of diffusion-limited nutrients such as phosphorus. At the same time, BAT477 maintains root biomass in the deep horizon that is comparable to the deep genotype, DOR364. This deep root fraction is primarily tap root, which is likely better for facilitating water transport. Though BAT477 does not exhibit it to a great extent in this study, root architectural plasticity may also allow for greater flexibility of a given root system, where roots would branch more shallowly if under phosphorus deficiency, but would branch more deeply otherwise, to allow for increased water uptake.

Our results show that root system architecture has important implications for resource acquisition in heterogeneous environments. Specifically, tradeoffs to different strategies exist and are dependent upon the specific environment. This work also shows that root size, root localisation and root carbon allocation to specific root types are all effective means of altering root architecture. Genotypes that allocate roots to surface horizons, particularly basal roots, are better adapted to low phosphorus environments. Genotypes that allocate root biomass to deep horizons, particularly to the tap root, are better adapted to terminal drought environments. Genotypes with a dimorphic root system, such as BAT477, which can maintain adequate root biomass in both shallow and deep soil layers are adapted to environments where both water and phosphorus are co-limiting. This work has important implications for crop breeders hoping to improve yield in environments with multiple soil resource constraints.

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