*Annals of Botany* **112**: 973–982, 2013
doi:10.1093/aob/mct164, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

# Basal root whorl number: a modulator of phosphorus acquisition in common bean (*Phaseolus vulgaris*)

**M. A. Miguel[1], A. Widrig[1], R. F. Vieira[1,2], K. M. Brown[1] and J. P. Lynch[1,*]**

[1]*Department of Plant Science, The Pennsylvania State University, University Park, PA 16802, USA and* [2]*Empresa de Pesquisa Agropecuária de Minas Gerais, Caixa Postal 216, CEP 36571-000 Viçosa, MG, Brazil*
** For correspondence. E-mail: JPL4@psu.edu*

Received: 21 February 2013 Returned for revision: 1 May 2013 Accepted: 3 June 2013 Published electronically: 7 August 2013

• *Background and Aims* Root architectural phenes enhancing topsoil foraging are important for phosphorus acquisition. In this study, the utility of a novel phene is described, basal root whorl number (BRWN), that has significant effects on topsoil foraging in common bean (*Phaseolus vulgaris*).
• *Methods* Whorls are defined as distinct tiers of basal roots that emerge in a tetrarch fashion along the base of the hypocotyl. Wild and domesticated bean taxa as well as two recombinant inbred line (RIL) populations were screened for BRWN and basal root number (BRN). A set of six RILs contrasting for BRWN was evaluated for performance under low phosphorus availability in the greenhouse and in the field. In the greenhouse, plants were grown in a sand–soil media with low or high phosphorus availability. In the field, plants were grown in an Oxisol in Mozambique under low and moderate phosphorus availability.
• *Key Results* Wild bean accessions tended to have a BRWN of one or two, whereas cultivated accessions had BRWN reaching four and sometimes five. BRWN and BRN did not vary with phosphorus availability, i.e. BRWN was not a plastic trait in these genotypes. Greater BRWN was beneficial for phosphorus acquisition in low phosphorus soil. Genotypes with three whorls had almost twice the shoot biomass, greater root length and greater leaf area than related genotypes with two whorls. In low phosphorus soil, shoot phosphorus content was strongly correlated with BRWN ($R^2 = 0{\cdot}64$ in the greenhouse and $R^2 = 0{\cdot}88$ in the field). Genotypes with three whorls had shallower root systems with a greater range of basal root growth angles (from 10 to 45 ° from horizontal) than genotypes with two whorls (angles ranged from 60 to 85 ° from horizontal).
• *Conclusions* The results indicate that BRWN is associated with increased phosphorus acquisition and that this trait may have value for selection of genotypes with better performance in low phosphorus soils.

**Key words:** Basal root whorls, phosphorus acquistion, common bean, *Phaseolus vulgaris*, root architecture.

## INTRODUCTION

Low phosphorus availability is a primary constraint for crop productivity in most tropical soils of Africa, Latin America and Asia (Vance *et al.*, 2003; Lynch, 2007). For example, 85 % of all common bean (*Phaseolus vulgaris*) areas in southern Africa are affected by low phosphorus availability (Lynch, 2007). Crop genotypes differ in phosphorus acquisition under low phosphorus availability (Lynch, 2007). Root architecture plays an important role in phosphorus acquisition because of spatial variation in soil phosphorus availability resulting from its low mobility, and factors related to phosphorus availability, such as soil pH, microbial activity and colloid chemistry (Lynch, 2011). The movement of phosphorus in soils is largely dependent on diffusion, so the plant itself contributes to the spatial heterogeneity of phosphorus by depleting it from the rhizosphere. For annual crop species that have relatively rapid growth, this necessitates continual exploration of new soil domains not already depleted of phosphorus by root activity. Root architecture determines the exploration and exploitation of localized phosphorus resources by the plant, and the distribution of roots relative to their neighbors within and among root systems, and is therefore an important component of phosphorus acquisition. Several root traits are related to plant adaptation to low phosphorus availability, including mycorrhizal symbioses (Glick *et al.*, 1999; Smith *et al.*, 1999), root morphological phenes including root hair length and density (e.g. Bates and Lynch, 1996; Ma *et al.*, 2001), and exudation of phosphorus-mobilizing compounds such as protons, organic acids and phosphatases (Ryan *et al.*, 2001; Hinsinger, 2011), but these processes are themselves distributed in the soil by root architecture. Root exudates are localized to the microenvironments determined by root distribution and, therefore, root architecture. Root architecture may, therefore, be viewed as a higher order organismic trait within which traits at the organ, tissue and cellular level operate (Lynch, 1995; Lynch and Brown, 2001).

Plants under phosphorus stress cannot simply grow more roots throughout the soil profile without diverting resources from shoot growth and grain production. The phosphorus costs of root growth may be relatively greater than the phosphorus costs of leaf growth, since, unlike leaves, roots appear to be unable to remobilize phosphorus effectively to the rest of the plant through programmed organ senescence (Snapp and Lynch, 1996). The optimal root architecture for phosphorus acquisition is therefore one which enhances phosphorus acquisition at minimum carbon cost, or optimizes the value of phosphorus gained with respect to the relative value of the resources required for root growth, including phosphorus itself (Lynch and Ho, 2005).

In this study, we focus on a novel root phene, basal root whorl number (BRWN), that may affect phosphorus acquisition in annual dicots such as common bean. The bean root system consists of a primary root, a variable number of basal roots originating from the basal portion of the hypocotyl, hypocotyl-borne roots (emerging from the sub-terranean hypocotyl) and lateral roots developing from each of the other root classes (Zobel, 1986; Lynch and van Beem, 1993). Basal roots in common bean emerge along the base of hypocotyls from distinct tiers (Basu *et al.*, 2007). We have termed these tiers or positions 'basal root whorls' (Fig. 1). Typically four roots emerge from each whorl. We hypothesize that genotypes with greater BRWN will have more dispersed basal roots, i.e. a greater range of growth angles. Shallow roots arising from the uppermost whorls would function for topsoil exploration and phosphorus acquisition, while deeper roots from the lower whorls would be important for water acquisition from drying soil. In this study, we attempt to determine the functional role of BRWN for phosphorus acquisition.

## MATERIALS AND METHODS

### *Greenhouse experiment*

*Plant materials.* The accessions of *Phaseolus vulgaris* used for evaluation for BRWN in the lab were obtained from parental and elite lines developed by CIAT headquarters in Cali, Colombia. They included common bean accessions as well as wild and cultivated taxa. For greenhouse and field experiments, two sets of recombinant inbred lines (RILs) of common bean (*P. vulgaris* L.) were used. One set of RILs was developed from G19833 and DOR 364 parents. G19833 is a Peruvian landrace (of the Andean gene pool) with an indeterminate bush prostrate growth habit (type III growth habit), observed in previous studies as having root characteristics conferring phosphorus acquisition efficiency. These characteristics include the ability to form longer and denser root hairs under low phosphorus availability (Yan *et al.*, 2004), formation of shallow basal roots suitable for phosphorus uptake from upper soil horizons where phosphorus is usually concentrated (Bonser *et al.*, 1996) and high BRWN (Basu *et al.*, 2004). DOR 364 is a high yielding line developed by bean breeders at CIAT (Cali, Colombia), and bred for resistance to *Bean golden yellow mosaic virus*. However, despite its high yielding characteristics, this genotype has been demonstrated to be phosphorus inefficient due to its root characteristics (Liao *et al.*, 2004). The other set of RILs were developed from the cross of G2333 × G19839. G2333 ('Colorado de Teopisca') is a climbing, small red-seeded Mexican landrace belonging to the Mesoamerican gene pool (Singh *et al.*, 1991) with a type IV growth habit (Singh *et al.*, 1995), and G19839 is a large, yellow and red-mottled seeded Peruvian landrace with type III growth habit from the Andean gene pool (Singh *et al.*, 1991). These two phosphorus-efficient landraces have contrasting phenotypes for a number of root traits, including BRWN, hypocotyl-borne rooting, and root hair length and density (Miller *et al.*, 2003; Walk *et al.*, 2004; Ochoa, 2006).

The RILs used in the experiment were selected to have contrasting BRWN but otherwise similar root phenotypes, in order to be able to associate differences in plant performance with variation in BRWN.

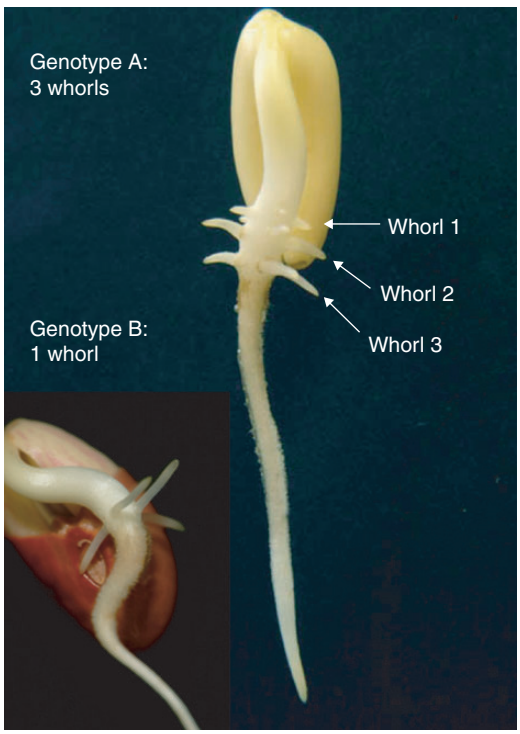


FIG. 1. Image of contrasting bean genotypes, showing basal roots arising from distinct whorls.

*Germplasm screening.* We evaluated 246 wild and cultivated bean accessions, and RILs of two populations of common bean for BRWN (see Supplementary Data Table S2). Seeds were surface sterilized with 0·5 % NaOCl for 1 min before being scarified and placed onto low phosphorus brown germination paper (Anchor Paper, St. Paul, MN, USA) saturated with 0·5 mM $CaSO_4$. Five seeds of each genotype were placed 2 cm from the top of a 20 cm long piece of germination paper, rolled into a moderately tight 'cigar roll' configuration and placed in a 1 L beaker with 100 mL of 0·5 mM $CaSO_4$. The beakers were filled with 12–13 rolls before being wrapped with cellophane, punctured with holes to allow aeration. The beakers were then placed into a germination chamber for 4–5 d at 28 °C. The BRWN and basal root number (BRN) per seedling were then recorded.

*Genotype screening for the greenhouse experiment.* Two RIL populations of common bean (*P. vulgaris* L.) were screened prior to the greenhouse study. One RIL population, composed of 76 RILs, was derived from DOR 364 × G19833, and the other population was from G2333 × G19839, and was composed of 57 RILs. During the screening, six seeds from each RIL were surface sterilized for 1–2 min in 10 % (v/v) NaOCl, thoroughly rinsed with de-ionized water, mechanically scarified, germinated in rolls of brown germination paper (Anchor Paper Co.), and then placed upright in 500 mL beakers containing 200 mL of 0·5 mM $CaSO_4$, and left to germinate for 3 d before evaluating for BRWN and BRN. Out of the large number of screened materials, we selected six contrasting genotypes for BRWN. Of these genotypes, three have three basal root whorls, while the other three genotypes have two basal whorls.

They were all from the G19839 × G2333 RIL population. They are similar in all other characteristics, such as seed size and colour, growth habit, days to flowering, root hair formation, etc., and the only apparent contrasting characteristic was BRWN. The selected genotypes were used in the experiment in the greenhouse.

*Growth media.* A mixture of 50 % commercial grade medium size yellow sand, 40 % D3 coarse vermiculite (Whittemore Co., Inc., Lawrence, MA, USA) and 10 % red soil (C horizon of Hagerstown silt loam, fine, mixed mesic, typic Hapludalf) was used as the growth medium. The red soil was used for its oxide surfaces that absorb and desorb phosphate and create diffusion-limited phosphate availability. Seeds were planted in 20 L opaque pots 10 cm in radius and 25 cm in height, wrapped with white duct tape to enhance reflectiveness. Nutrients were supplied through the irrigation system with 4 μM $KH_2PO_4$ for low phosphorus treatments and 500 μM $KH_2PO_4$ for high phosphorus treatments. The other nutrients were: 1·5 mM $KNO_3$, 1·2 mM $Ca(NO_3)_2$, 0·4 mM $NH_4NO_3$, 0·025 mM $MgCl_2$, 0·5 mM $MgSO_4$, 0·3 mM $K_2SO_4$, 0·3 mM $(NH_4)_2SO_4$, 5 μM Fe-EDTA, 1·5 μM $MnSO_4$, 1·5 μM $ZnSO_4$, 0·5 μM $CuSO_4$, 0·15 μM $(NH_4)_6Mo_7O_{24}$ and 0·5 μM $Na_2B_4O_7$. The pH of the nutrient solution was adjusted every other day to 5·8 with KOH and HCl. Nutrients were supplied through the irrigation system twice daily. Each irrigation event supplied 20·8 mL of nutrient solution at the seedling stage, and, starting at 4 weeks after planting, the amount was increased up to 30–45 mL per irrigation.

*Experimental design.* A completely randomized design was used in the greenhouse experiment, with two phosphorus levels: low phosphorus (4 μM $KH_2PO_4$) and high phosphorus (500 μM $KH_2PO_4$). Each treatment had four replications of three genotypes per BRWN class. The experiment was conducted under controlled conditions in a greenhouse at The Pennsylvania State University, University Park, PA, USA (40 °85′N, 77 °82′W), during June–August 2006. The average temperature was 26 °C, mid-day photosynthetic active radiation (PAR) averaged 800–1000 μmol photons $m^{-2}$ $s^{-1}$, and the average humidity was 60 %. Natural light was supplemented from 0800 to 2000 h with 110 μmol photons $m^{-2}$ $s^{-1}$ from 400 W metal halide bulbs (Energy Technics, York, PA, USA).

*Data collection and analysis.* Data collected in the greenhouse experiment included total shoot dry weight, shoot tissue phosphorus content, total root length, BRWN and BRN. Shoot tissue included ground leaves and stems of the plant. Plant samples were collected at 14, 21 and 28 d after planting. Plant shoots were collected, placed in paper bags and dried at 60 °C for 3 d before recording shoot dry weight. Total root length data were collected by first washing roots in water, then preserving them in 25 % ethanol. Total root length was determined by image analysis (WinRhizo Pro, Régent Instruments, Québec, Canada). Images of the root system were taken by scanning the whole root system, using an EPSON Perfection V700 PHOTO scanner with image DPI 23·6 pixels $mm^{-1}$, which showed enough clarity to identify and quantify fine roots using the WinRhizo software (Fig. 2). The BRWN and BRN were determined by counting the root whorls and basal roots after washing the root system and before preservation. Tissue phosphorus content was determined spectrophotometrically (Murphy and Riley, 1962) after ashing at 500 °C for 12 h. Analysis of variance (ANOVA), Fisher's test for comparisons between means, and regression analysis were performed using Minitab 15 (Minitab Inc., State College, PA, USA).

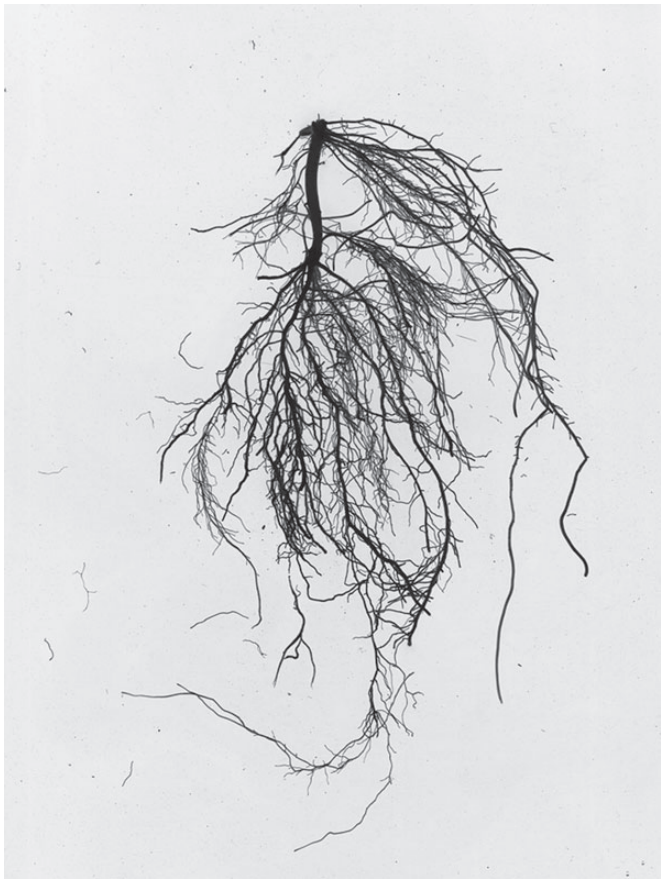

FIG. 2. Image of a scanned root system using an EPSON Perfection V700 scanner at image resolution in DPI of 23·6 pixels $mm^{-1}$. WinRhizo software used the root system images for root system analysis.

### *Field experiment*

*Plant materials.* The contrasting parental lines G2333 and G19839 and four RILs derived from them were used in the field experiment. The genotypes used in this study were grouped in two root phenotype categories: two whorls (G2333, GG37 and GG80) and three whorls (G19839, GG41 and GG48). We selected RILs from this population because we observed a smaller coefficient of variation within a phene category among the RILs from the G2333 × G19839 population compared with the DOR 364 × G19833 population.

*Experimental design.* A completely randomized design was used in the field experiment. Treatments were established under low and medium phosphorus availability. Low phosphorus had 6 ppm available phosphorus (Olsen), while medium phosphorus treatments had 19 ppm available phosphorus (Olsen). Each treatment had four replications of each of three genotypes per BRWN phenotype (two vs. three whorls). Each plot contained three rows 2 m in length with 60 cm between rows. Twenty-one seeds were

planted in each row, with a spacing of 10 cm within the row (60 × 10 cm spacing).

*Field characteristics.* The field study was carried out in Mozambique at the Sussundenga Research station in Manica Province (19 °19′02·00″S and 33 °14′25·24″E, 620 ma.s.l.). The soil type at the research site is an Ustox with low pH (4·5–5·5). Three months before planting, the soil was limed ($CaCO_3$) to bring the soil pH to 6·2. The annual average precipitation is 1100 mm. However, in the year of this study, the region experienced some drought during the growing season, and the annual precipitation was about 758 mm, unevenly distributed, so the field was irrigated as needed to keep soil moisture content close to field capacity. Temperatures ranged from 14 to 28 °C. The experiment was planted in February 2010. Seeds were inoculated with *Rhizobium* inoculum (Bunda College Microbiology Lab, Malawi) on the day of planting. The experiment had high and low phosphorus treatments. All other nutrients were kept optimal through chemical fertilization. Simple superphosphate was used as the source of additional phosphorus for fertilized plots applied at the rate of 100 kg of $P_2O_5$ $ha^{-1}$. After harvest, fertilized plots had 19 ppm and low phosphorus plots had 5·5 ppm available phosphorus, indicating that we had medium and low phosphorus treatments. This phosphorus content was stratified in the soil profile, with greater phosphorus in the top 15 cm with a rapid decline below 15 cm depth in both low and medium phosphorus plots. Weed control was performed manually. Pesticides were applied as needed.

*Data collection and analysis.* Data collected included shoot dry weight, total root length, total leaf surface area, BRWN, BRN and total phosphorus content. Plant samples were collected in three harvests at 14, 21 and 28 d after planting. Shoot biomass was determined from samples dried at 60 °C for 5 d. Tissue phosphorus content was determined spectrophotometrically (Murphy and Riley, 1962). During shoot sampling, leaf discs (6·6 $cm^2$) were collected from five fully expanded leaves. The ratio of dry weight to area of these discs was used to estimate total leaf area from total leaf dry weight. At plant sampling, root crowns were excavated and placed in a 20 L container with soap for washing. Detergent was added to the water used to wash the roots which helped to separate roots from soil particles without significantly damaging the root system or causing the loss of a large number of fine roots. Then root samples were washed and rinsed in clean tap water and placed in the vials with 25 % ethanol solution for preservation. Roots were scanned on an EPSON Perfection V700 PHOTO scanner from ICE digital technologies (Epson UK Ltd, Hemel Hempstead, UK). The images were analysed for total root length and root length by root diameter class using WinRhizo Pro at image DPI of 23·6 pixels $mm^{-1}$. In order to evaluate root distribution with soil depth, we took cores in the field at 28 d after planting. Root coring consisted of extracting soil samples by hammering a 5 cm diameter metal cylinder vertically into the soil. Cores were separated into 0–15 and 15–30 cm soil depths. Root fragments were recovered from each of these soil sections and analysed using WinRhizo Pro for root length determination. Although genotypes were selected based on their BRWN, plants harvested at 14 d after planting were re-assessed for BRWN to confirm the root phenotypes. The evaluation consisted of selecting and excavating three representative plants from each replication, determining BRWN and BRN by counting root whorls and basal roots, and calculating the average value for each replicate. ANOVA, Tukey's test for comparisons between means and regression analysis were conducted using Minitab (State College, PA, USA).

## RESULTS

### *Germplasm screening*

The number of basal roots was related to the number of whorls, with approximately four basal roots per whorl. A total of 246 *Phaseolus* taxa were screened for BRWN, BRN and seed weight. By the third day of germination in the growth chamber, all whorls had formed and basal roots had emerged. Wild accessions had 1–2 whorls and 4–8 basal roots, while cultivated taxa had 2–4 basal root whorls and 8–16 basal roots (Fig. 3). Six out of 35 CIAT parents and elite lines screened for BRWN and BRN had three whorls and 12 basal roots, while the remaining lines had an average of two basal root whorls and eight basal roots (Table 1). Screening data from all the materials show that there is no correlation between seed weight and BRWN in *P. vulgaris*, although there is a correlation in other *Phaseolus* species ($R^2 = 0{\cdot}48$). The lack of correlation was also observed from screening of Andean and Mesoamerican gene pools that differ in seed size, but with no significant correlation to BRWN. Although BRWN varied from one to four, most genotypes had two whorls (Table 2). Upper whorls formed basal roots with shallower growth angles, with an increase in angle in the lower whorls, leading to the formation of deeper roots from the lower whorls (Table 3). Greenhouse studies showed that phenotypes with two whorls had a greater percentage of reduction of total root length compared with phenotypes with three whorls (Table 4). Whorl effect was statistically significant in plants growing under low phosphorus availability (Table 5). Significant genotypic variation in BRWN was observed among RILs from both DOR364 × G19833 and G2333 × G19833 populations. The BRWN was closely correlated with the BRN.

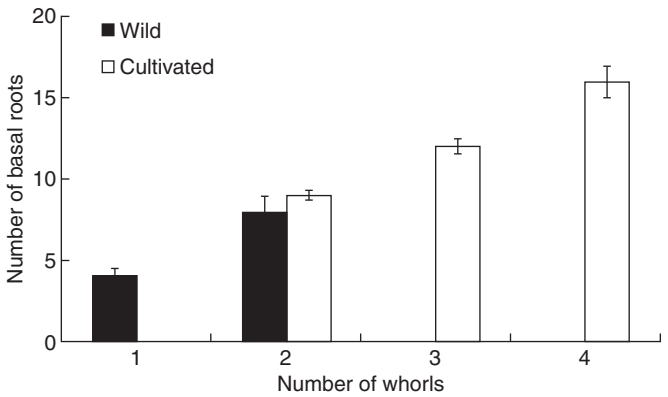


FIG. 3. Variation of basal root whorl number and basal root number in 246 wild and cultivated common bean species at the seedling stage. Sixty-four wild taxa and 182 cultivated taxa were evaluated for BRWN and BRN. Error bars represent the s.e. The value of each species was a mean of six seeds screened 3 d after germination in the laboratory.

Genotypes with greater BRWN had a greater range of growth angles of basal roots than genotypes with fewer whorls. Additional information about the variation in basal root whorl number, basal root number and seed weight can be viewed in Supplementary Data Table S1.

*Greenhouse experiment*

Correlation analysis among all accessions used in the study revealed that BRWN varied among genotypes regardless of seed weight, especially in the cultivated *Phaseolus* taxa. Under low phosphorus availability, there was a strong positive

TABLE 1. *Screening of CIAT parents and elite lines for BRWN and BRN*

| Genotype | Average BRWN | Standard deviation (BRWN) | Average BRN | Standard deviation (BRN) | Average seed weight (g) |
|---|---|---|---|---|---|
| G4494 | 3·2 | 0·02 | 12·0 | 2·50 | 0·58 |
| CAL125 | 3·0 | 0·0 | 12·0 | 0·00 | 0·40 |
| G14655 | 3·0 | 0·0 | 11·2 | 0·79 | 0·49 |
| CERINZA | 3·0 | 0·0 | 10·0 | 0·71 | 0·52 |
| CAL149 | 3·0 | 0·0 | 10·2 | 0·84 | 0·53 |
| ZPV292 | 3·0 | 0·0 | 9·6 | 0·89 | 0·36 |
| TLP19 | 2·8 | 0·44 | 10·4 | 1·52 | 0·24 |
| G19842 | 2·6 | 0·45 | 9·8 | 1·79 | 0·42 |
| CRF61 | 2·6 | 0·54 | 9·2 | 1·30 | 0·24 |
| MAR*1 | 2·6 | 0·54 | 9·0 | 1·00 | 0·25 |
| CARIOCA | 2·4 | 0·50 | 8·8 | 1·10 | 0·16 |
| AND696 | 2·2 | 0·44 | 8·6 | 1·34 | 0·45 |
| VAX1 | 2·2 | 0·44 | 8·4 | 0·89 | 0·22 |
| BF29 | 2·0 | 0·0 | 8·0 | 0·00 | 0·23 |
| BF19 | 2·0 | 0·0 | 8·0 | 0·00 | 0·24 |
| DICTA17 | 2·0 | 0·0 | 8·0 | 0·00 | 0·32 |
| BF54 | 2·0 | 0·0 | 8·0 | 0·00 | 0·27 |
| TLP35 | 2·0 | 0·0 | 8·0 | 0·00 | 0·23 |
| BF49 | 2·0 | 0·0 | 8·0 | 0·00 | 0·24 |
| MD2324 | 2·0 | 0·0 | 8·0 | 0·00 | 0·23 |
| VAX6 | 2·0 | 0·0 | 8·0 | 0·00 | 0·19 |
| BAT881 | 2·0 | 0·0 | 8·0 | 0·00 | 0·17 |
| SEA5 | 2·0 | 0·0 | 8·0 | 0·00 | 0·23 |
| RAB651 | 2·0 | 0·0 | 8·0 | 0·00 | 0·20 |
| BAT477 | 2·0 | 0·0 | 8·0 | 0·00 | 0·17 |
| A774 | 2·0 | 0·0 | 8·0 | 0·00 | 0·41 |
| G4825 | 2·0 | 0·0 | 8·0 | 0·00 | 0·22 |
| G19227A | 2·0 | 0·0 | 8·0 | 0·00 | 0·22 |
| AFR475 | 2·0 | 0·0 | 8·0 | 0·00 | 0·20 |
| SEQ7 | 2·0 | 0·0 | 8·0 | 0·00 | 0·27 |
| AND774 | 2·0 | 0·0 | 8·0 | 0·00 | 0·41 |
| MAM38 | 2·0 | 0·0 | 8·0 | 0·00 | 0·28 |
| RAB665 | 2·0 | 0·0 | 8·0 | 0·00 | 0·26 |
| G3513 | 2·0 | 0·0 | 8·0 | 0·00 | 0·23 |
| G21212 | 2·0 | 0·0 | 8·0 | 0·00 | 0·23 |

Average whorl and basal root number are based on the screening of five replicates per genotype.

TABLE 3. *Variation in basal root angles among root whorls of shallow-rooted and deep-rooted genotypes in the field experiment*

| Whorl position* | Range of basal root growth angle (degrees from horizontal) | |
|---|---|---|
| | Two-whorl phenotype | Three-whorl phenotype |
| Whorl 4 | 5–15 | 0–10 |
| Whorl 3 | 15–85 | 10–35 |
| Whorl 2 | – | 35–55 |
| Whorl 1 | – | – |
| Mean of root angles | 44·8 | 23 |

Ranges in angle were obtained from 24 plants (six contrasting genotypes in four replications in low phosphorus treatments). Genotypes were considered shallow rooted if the average angle (measured from root to the horizontal line) of all the whorls was $<45^{\circ}$, and deep rooted if the average angle of all whorls was $\geq 45^{\circ}$ from horizontal. Means of root angles were calculated by summation of the root angles value recorded in all evaluated genotypes in each phene category divided by the number of observations.

* Whorl position was counted from basipetal to acropetal positions.

TABLE 4. *Reduction of total root length by low phosphorus treatment among genotypes from the two whorl classes*

| BRWN class | Genotype | Total root length, m per plant | | % Reduction | Class mean |
|---|---|---|---|---|---|
| | | LP | HP | | |
| 2 whorls | DG76 | 120·52 | 296·02 | 59·3[a] | |
| 2 whorls | DG73 | 130·77 | 313·43 | 58·3[a] | |
| 2 whorls | DG13 | 145·53 | 391·05 | 62·8[a] | 60·1[a] |
| 3 whorls | DG62 | 179·59 | 308·00 | 41·7[b] | |
| 3 whorls | DG51 | 206·72 | 391·04 | 47·1[ab] | |
| 3 whorls | DG43 | 232·85 | 420·40 | 44·6[b] | 44·5[b] |

Root length values are means of four plants grown in the greenhouse and harvested at 28 d after planting.

Values followed by same letter within a column are not statistically different. Significant differences in percentage of total root length reduction were observed between BRWN classes.

TABLE 2. *Summary of BRWN and BRN in 173* P. vulgaris *accessions*

| Genotype | BRWN class | No. of genotypes | Basal root number | Seed weight (g) |
|---|---|---|---|---|
| G19833 × DOR 364 RILs and parents | 2 | 69 | 8·09 (±0·40) | 0·271(±0·052) |
| | 3 | 19 | 10·59 (±0·82) | 0·290 (±0·064) |
| G19839 × G2333 RILs and parents | 2 | 49 | 8·04 (0·25) | 0·355 (±0·064) |
| | 3 | 11 | 10·56 (±0·73) | 0·398 (±0·052) |
| CIAT parents and elite lines | 2 | 24 | 8·04 (±0·32) | 0·255 (±0·073) |
| | 3 | 10 | 10·44 (±0·71) | 0·403 (±0·128) |
| Wild *P. vulgaris* | 2 | 52 | 7·34 (±0·79) | 0·066 (±0·037) |
| | 1 | 12 | 4·77 (±0·99) | 0·046 (±0·017) |

For BRWN class, genotypes were classified as three-whorl if they had more than an average of 2·5 whorls or as two-whorl if they had fewer than 2·5 whorls. Numbers in parentheses represent the s.e.

TABLE 5. *Shoot phosphorus content in BRWN classes of common bean genotypes grown under low phophorus (5 ppm) and medium phosphorus (19 ppm) in the field*

| | Shoot phosphorus content, mg per plant | |
|---|---|---|
| Phosphorus treatment | 2 whorls | 3 whorls |
| Low phosphorus | 3·84 (±0·08) | 7·18 (±0·18) |
| Medium phosphorus | 7·52 (±0·38) | 11·25 (±0·21) |

Each BRWN class was composed of three genotypes, making a total of six genotypes tested, replicated four times. Values shown are means ± s.e., for four replicates.

Whorl effects in both low and medium phosphorus treatments were statistically significant at $P = 0{\cdot}05$ with an $F$-value equal to 133·97, according to Tukey's test used for mean comparisons.

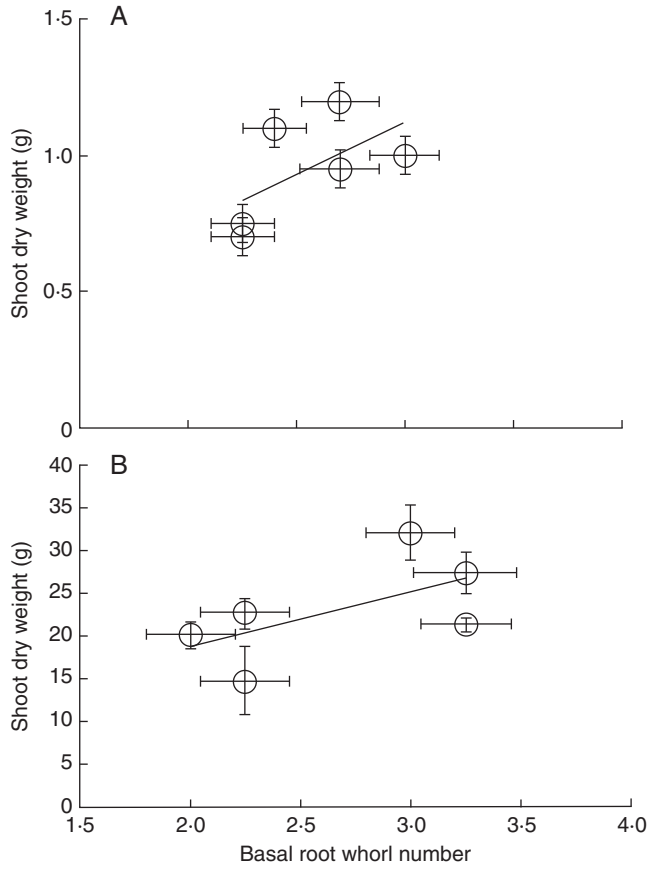


FIG. 4. Correlation between shoot dry weight and basal root whorl number in six genotypes of common bean grown under contrasting phosphorus availability in greenhouse conditions. A strong positive correlation ($R^2 = 0{\cdot}64$) was observed at 28 d after planting in low phosphorus (A) and a positive correlation ($R^2 = 0{\cdot}36$) was found in high phosphorus (B) plants grown in the greenhouse. Each point is the average of four replicates; error bars represent the s.e.

correlation between BRWN and shoot dry weight (Fig. 4) and shoot phosphorus content (Fig. 5). There was no correlation between hypocotyl-borne roots and BRWN and BRN, regardless of phosphorus treatment. Genotypes with less BRWN had a greater percentage reduction in phosphorus content compared with genotypes with greater BRWN, when grown under low

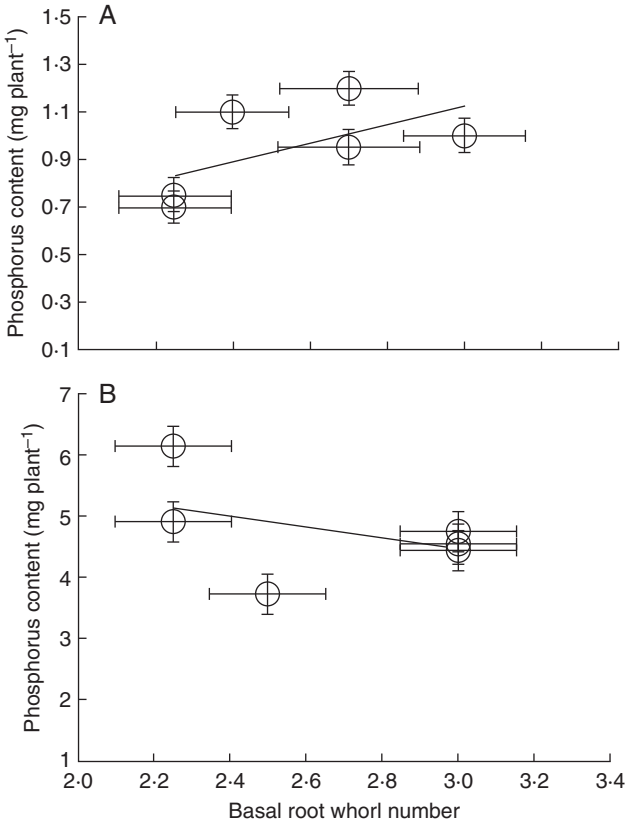


FIG. 5. Correlation between shoot phosphorus content and BRWN in six contrasting genotypes grown under low (A) and high (B) phosphorus availability in the greenhouse. Each point is the average of four replicates; error bars represent the s.e. A significant correlation was found under low phosphorus ($R^2 = 0{\cdot}63$), while under high phosphorus the correlation was not significant ($R^2 = 0{\cdot}18$).

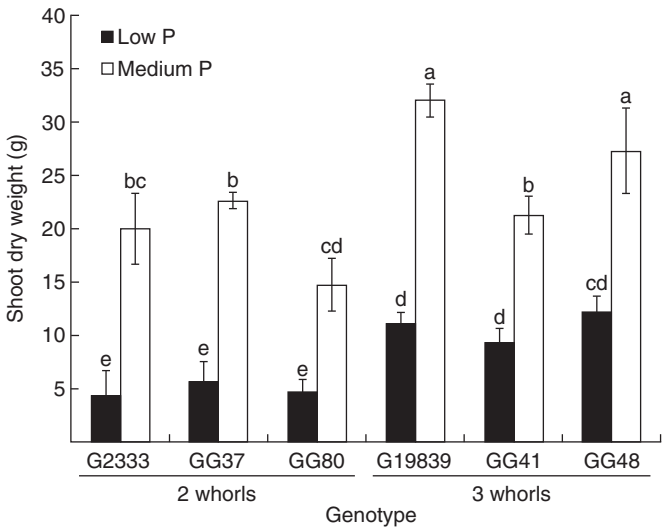


FIG. 6. Shoot dry weight of six genotypes contrasting for BRWN, grown under low (5 ppm) or medium phosphorus (19 ppm) in the field. Plant samples were collected at 28 d after planting. Each point is the average of four replicates; error bars represent the s.e. Means with the same letter are not significantly different at $P = 0{\cdot}05$. Under low phosphorus treatment, genotypes with three whorls had greater shoot dry weight compared with two-whorled genotypes.

phosphorus availability. Low phosphorus reduced root length by 60·1 % in two-whorl genotypes, vs. 44·5 % in three-whorl genotypes.

*Field experiment*

The BRWN and BRN phenotypes in the field were consistent with seedling screening data. Genotypes G2333, GG37 and GG80 had two whorls and eight basal roots while genotypes G19839, GG41 and GG48 had three whorls and 12 basal roots on average. Under low phosphorus, three-whorl genotypes had greater shoot dry weight than two-whorl genotypes (Fig. 6), as well as greater leaf area and greater phosphorus content (Fig. 7). The BRWN affected phosphorus content in both low and medium phosphorus (Table 6).

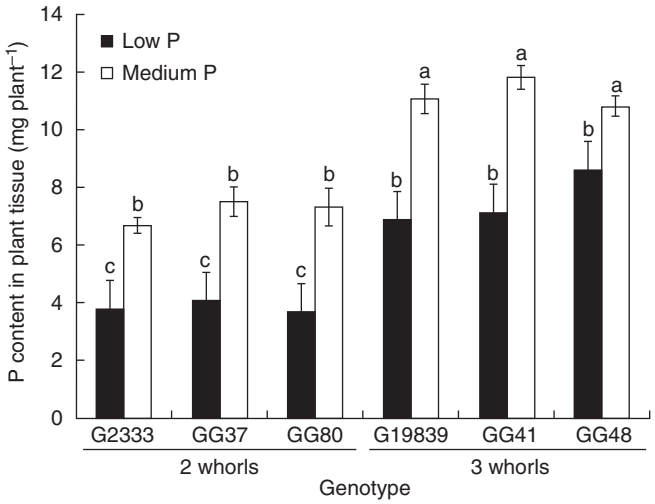


FIG. 7. Phosphorus content among six genotypes contrasting for BRWN grown under low (5 ppm) and medium phosphorus (19 ppm) in the field. Each point is the average of four replicates; error bars represent the s.e. Means with the same letter are not significantly different at $P = 0{\cdot}05$.

Genotypes were also evaluated for root hair length. All genotypes had greater root hair length under low phosphorus availability compared with medium phosphorus availability, but BRWN was unrelated to root hair length (Table 6). However, for phenotype GG48, root hair length did not vary with phosphorus treatment, and it was significantly lower compared with other genotypes under low phosphorus.

We collected soil cores at two depths in the field at 28 d after planting to analyse root length distribution with depth. Genotypes with three whorls had more roots in the top 15 cm of soil than genotypes with two whorls, while genotypes with two whorls had greater root length in the 15–30 cm segments (Fig. 8). Total root length from soil cores (0–30 cm depth) was greater in genotypes with three whorls compared with genotypes with two whorls. This was confirmed in scans of the entire root cores, which showed that genotypes with three whorls had greater root length compared with genotypes with two whorls under both low and medium phosphorus availability (Fig. 8). Differences in the number of hypocotyl-borne roots were observed among genotypes, but they were not influenced by phosphorus availability (Fig. 9). In addition, variation in hypocotyl-borne rooting was not related to variation in BRWN among phenotypes, which shows that BRWN is independent from hypocotyl-borne rooting.

## DISCUSSION

Our results are consistent with the hypothesis that greater BRWN is a positive adaptation to low soil phosphorus availability. Under low phosphorus availability in the field and in controlled conditions in the greenhouse, bean genotypes with three basal root whorls had substantially greater shoot dry weight, shoot phosphorus content and leaf area than genotypes with two whorls. The utility of BRWN for phosphorus acquisition is probably

TABLE 6. *Results of linear models on the effects of BRWN and phosphorus treatments on shoot growth and root development in the field experiment*

| Variable | | Phene | Phosphorus level | Phene vs. phosphorus level | $R^2$ |
|---|---|---|---|---|---|
| Shoot dry weight, g | d.f. | 1 | – | 1 | 0·91 |
| | *F*-ratio | 10·20** | 49·85*** | 0·43 | – |
| Total leaf area, cm$^2$ per plant | d.f. | 1 | – | 1 | – |
| | *F*-ratio | 6·78 | 28·60*** | – | 0·86 |
| Phosphorus content, mg per plant | d.f. | 1 | 1 | 1 | 0·97 |
| | F-ratio | 133·97*** | 111·85*** | 0·34 | – |
| Root hair length, mm | d.f. | 1 | 1 | 1 | 0·90 |
| | *F*-ratio | 0·01 | 50·57 | – | – |
| Root core length at 0–15 cm depth, cm | d.f. | 1 | 1 | 1 | 0·95 |
| | *F*-ratio | 106·11*** | 0·66 | – | – |
| Root core length at 15–30 cm depth, cm | d.f. | 1 | 1 | 1 | 0·88 |
| | *F*-ratio | 276·43*** | 5·8 | – | – |
| Total core root length, cm | d.f. | 1 | 1 | 1 | 0·94 |
| | *F*-ratio | 83·01 | 3·4 | – | – |
| Basal root number | d.f. | 1 | 1 | 1 | 0·98 |
| | *F*-ratio | 392·39 | 1·09 | – | – |

'Phene' refers to the BRWN class, two whorls vs. three whorls.
Phosphorus level indicates the soil phosphorus availability: medium phosphorus (18 mg L$^{-1}$) and low phosphorus (5 mg L$^{-1}$).
Values are ignificant at ***$P \leq 0{\cdot}05$; **$P \leq 0{\cdot}01$; *$P \leq 0{\cdot}1$.

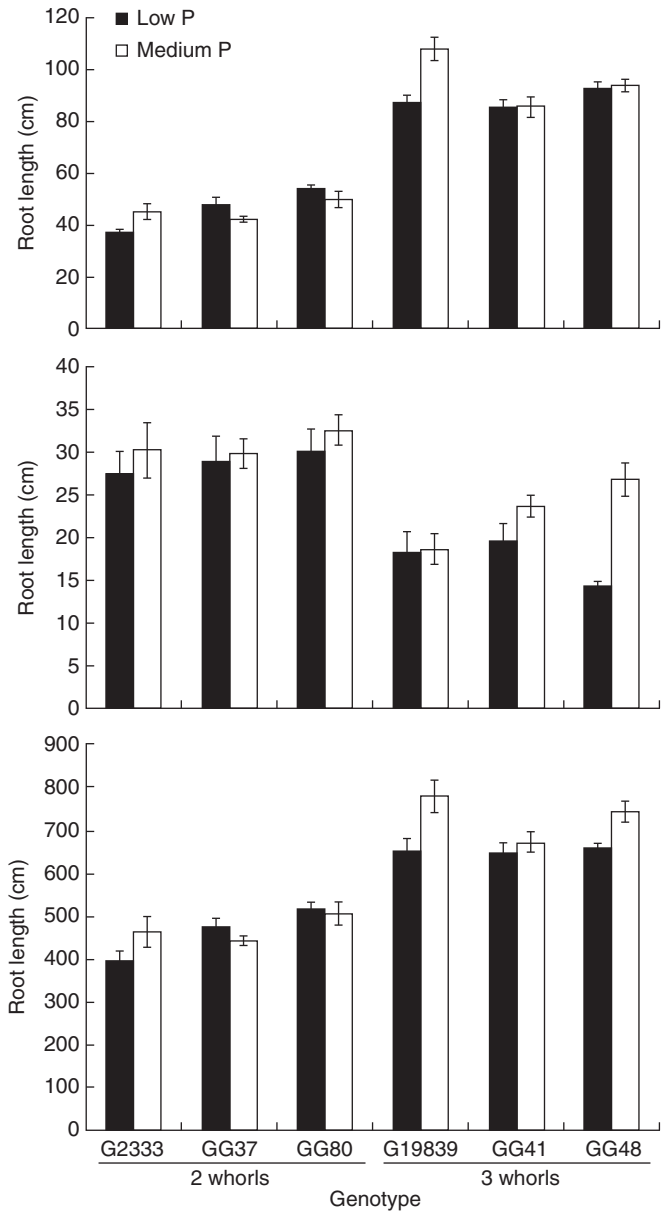


FIG. 8. Root length recovered from soil cores at 0–15 cm (top) and 15–30 cm depth of soil profile (centre), and total root core length (bottom). Root length (in cm) is per core volume at each depth of soil profile (see Table 6 ANOVA). Each point is the average of four replicates; error bars represent the s.e.

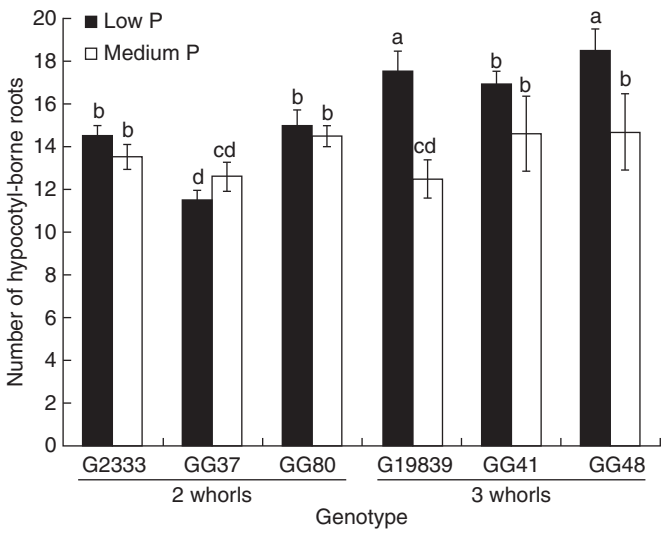


FIG. 9. Number of hypocotyl-borne roots in six common bean genotypes contrasting in BRWN. Plant samples were taken at 28 d after planting in a field experiment. Each point is the average of four replicates; error bars represent the s.e. Means with the same letter are not significantly different at $P = 0{\cdot}05$.

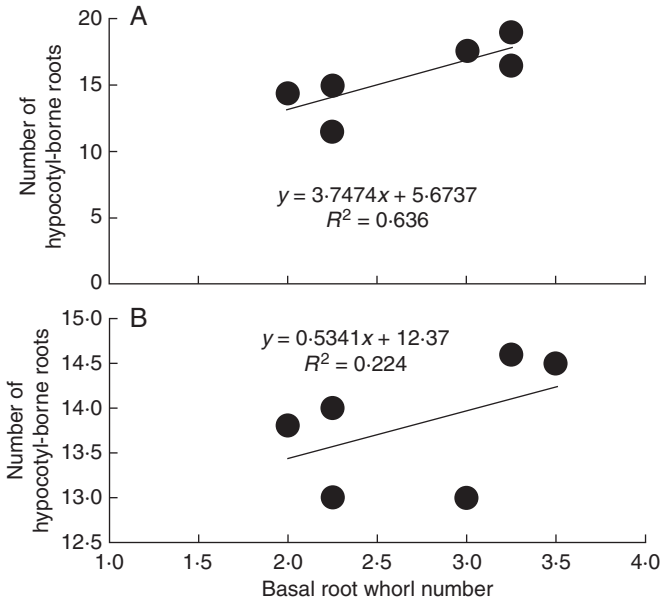


FIG. 10. Correlation between basal root whorl number and number of hypocotyl-borne roots among contrasting common bean genotypes grown under low (A) and medium (B) phosphorus availability.

due to greater soil exploration, since greater BRWN was associated with more basal roots, more root length and greater topsoil foraging, which is advantageous in phosphorus-limited environments since phosphorus availability in most soils is greatest in surface horizons (Lynch and Brown, 2001). Greater BRWN is beneficial not only by promoting an increase in BRN, but also by enabling a more dispersed root system that is capable of maximizing the soil volume exploited by the plant, as shown by the greater range of basal root growth angles in genotypes with greater BRWN (Basu *et al.*, 2007). Genotypes with three whorls have a greater vertical range of soil exploitation compared with genotypes with two whorls. Data from root cores showed that genotypes with three whorls had greater root length at 0–15 cm depth than at 15–30 cm depth, while genotypes with two whorls had greater root length at 15–30 cm than at 0–15 cm depth. However, genotypes with three whorls had greater total root length compared with genotypes with two whorls (Fig. 8). A broader vertical range of soil exploitation can be important for conditions in which critical resources are located in both shallow and deep soil domains, such as occurs in conditions of drought and low phosphorus availability.

We observed variation in the number of root whorls and basal roots among genotypes from two RIL populations and other genotypes, including wild and cultivated *Phaseolus* accessions. Phenotypic profiling of a wide range of wild and cultivated bean taxa showed that wild accessions have fewer basal root whorls, and consequently fewer basal roots compared with cultivated accessions. Greater BRWN in cultivated accessions may reflect plant adaptation to the less favourable environments that these accessions experienced with domestication, where crops were grown under continuous cultivation in soils subject to degradation, in soils beyond their initial range of adaptation.

In the field study, we did not have a truly high phosphorus treatment, but rather medium phosphorus, i.e. both phosphorus treatments were sub-optimal for plant growth. In soil with high phosphorus availability, variation for BRWN and BRN would not be expected to affect plant fitness by changing phosphorus acquisition. However, a greater number of large diameter axial roots such as basal roots may create trade-offs by diverting internal resources from competing uses such as axial elongation or branching, or shoot growth and reproduction. Such trade-offs between basal roots and hypocotyl-borne roots have been demonstrated in common bean (Walk *et al.*, 2006; Rubio and Lynch, 2007). In this study, we observed no correlation of hypocotyl-borne roots with variation in BRWN in medium phosphorus and a weak correlation in low phosphorus (Fig. 10). We did not observe compensatory reductions in hypocotyl-borne rooting in high BRWN phenotypes. In a study with common bean in which root classes were selectively removed, strong compensation was observed between the growth of basal and adventitious (i.e., hypocotyl-borne) roots (Rubio *et al.*, 2007). We did not observe a significant compensatory effect on adventitious roots with variation in BRWN and BRN. This may indicate that the processes responsible for compensation between basal and adventitious rooting are driven by lateral roots rather than axial roots. Our detailed comparisons were of two- vs. three-whorled phenotypes; it is possible that stronger compensatory effects and trade-offs would occur in more extreme phenotypes having four or more whorls. However, we observed an increase in the number of hypocotyl-borne roots in the low phosphorus treatment in all genotypes regardless of BRWN. This confirms earlier observations that low phosphorus availability increases hypocotyl-borne rooting in common bean (Miller *et al.*, 2003; Ochoa *et al.*, 2006). Trade-offs to high BRWN may be important under drought stress, as increased basal roots may reduce internal resources available to individual basal root axes, slowing elongation into deeper soil domains. Such trade-offs should be understood for informed deployment of this trait in plant breeding. BRWN appears promising as a phene for developing genotypes of bean and possibly other dicot crops with better productivity in low P soils.

BRWN appears to be an important trait for phosphorus acquisition in plants growing under low phosphorus availability. The process of germplasm screening for BRWN is relatively easy and straightforward. Seedlings can be non-destructively phenotyped for BRWN and BRN 3 d after germination using simple techniques in the lab.

Increased BRWN may also be beneficial for resistance to biotic stresses. A phenotype with greater BRWN also has greater BRN, providing greater total root length, root surface area, root branching and proliferation. These characteristics are important in circumstances where part of the root system is lost to herbivory or disease, as commonly occurs in low-input ecosystems.

## SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: summary of wild *P. vulgaris* BRWN and BRN screening results. Table S2: data from all the materials (wild, cultivated and breeding lines) included in the study.

## LITERATURE CITED

**Basu P, Zhang Y, Lynch J, Brown K. 2007.** Ethylene modulates genetic, positional, and nutritional regulation of root plagiogravitropism. *Functional Plant Biology* **34**: 41–51.

**Bates TR, Lynch JP. 1996.** Stimulation of root hair elongation in *Arabidopsis thaliana* by low phosphorus availability. *Plant, Cell and Environment* **19**: 529–538.

**Bonser AM, Lynch J, Snapp S. 1996.** Effect of phosphorus deficiency on growth angle of basal roots in Phaseolus vulgaris. *New Phytologist* **132**: 281–288.

**Glick B, Patten C, Holguin G, Penrose D. 1999.** *B*iochemical and genetic mechanisms used by plant growth promoting bacteria. London: Imperial College Press.

**Hinsinger P. 2001.** Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant and Soil* **237**: 173–195.

**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. *Functional Plant Biology* **31**: 1–12.

**Lynch JP. 1995.** Root architecture and plant productivity. *Plant Physiology* **109**: 7–13.

**Lynch JP. 2007.** Roots of the second green revolution. *Australian Journal of Botany* **55**: 493–512.

**Lynch JP. 2011.** Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. *Plant Physiology* **156**: 1041–1049.

**Lynch JP, van Beem JJ. 1993.** Growth and architecture of seedling roots of common bean genotypes. *Crop Science* **33**: 1253–1257.

**Lynch JP, Brown KM. 2001.** Topsoil foraging – an architectural adaptation of plants to low phosphorus availability. *Plant and Soil* **237**: 225–237.

**Lynch JP, Ho MD. 2005.** Rhizoeconomics: carbon costs of phosphorus acquisition. *Plant and Soil* **269**: 45–56.

**Ma Z, Bielenberg DG, Brown KM, Lynch JP. 2001.** Regulation of root hair density by phosphorus availability in *Arabidopsis thaliana*. *Plant, Cell and Environment* **24**: 459–467.

**Miller CR, 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. *Functional Plant Biology* **30**: 973–985.

**Murphy J, Riley JP. 1962.** A modified single solution method for the determination of phosphorus in natural waters. *Analytica Chimica Acta* **27**: 31–36.

**Ochoa I, Blair M, Lynch JP. 2006.** QTL analysis of adventitious root formation in common bean (*Phaseolus vulgaris* L.) under contrasting phosphorus availability. *Crop Science* **46**: 1909–1921.

**Rubio G, Lynch JP. 2007.** Compensation among root classes in *Phaseolus vulgaris* L. *Plant and Soil* **290**: 307–321.

**Ryan PR, Delhaize E, Jones DL. 2001.** Function and mechanism of organic anion exudation from plant roots. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**: 527–560.

**Singh SP, Gepts P, Debouck DG. 1991.** Races of common bean (*Phaseolus vulgaris*, Fabaceae). *Economic Botany* **45**: 379–396.

**Singh SP, Molina A, Gepts P. 1995.** Potential of wild common bean for seed yield improvement of cultivars in the tropics. *Canadian Journal of Plant Science* **75**: 807–813.

**Smith JD, Staehelin LA, Todd P. 1999.** Early root cap development and gravir-esponse in white clover (*Trifolium repens*) grown in space and on a two-axis clinostat. *Journal of Plant Physiology* **155**: 543–550.

**Snapp SS, Lynch JP. 1996.** Phosphorus distribution and remobilization in bean plants as influenced by phosphorus nutrition. *Crop Science* **36**: 929–935.

**Vance CP, Uhde-Stone C, Allan DL. 2003.** Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytologist* **157**: 423–447.

**Walk TC, Van Erp E, Lynch JP. 2004.** Modelling applicability of fractal analysis to efficiency of soil exploration by roots. *Annals of Botany* **94**: 119–128.

**Walk TC, Jaramillo R, Lynch JP. 2006.** Architectural tradeoffs between adventitious and basal roots for phosphorus acquisition. *Plant and 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 and Soil* **265**: 17–29.

**Zobel RW. 1986.** Rhizogenetics (root genetics) of vegetable crops. *HortScience* **21**: 956–959.