FUNCTIONAL ROLE AND SYNERGYSTIC EFFECT OF ROOT TRAITS FOR PHOSPHORUS ACQUISITION EFFICIENCY AND THEIR GENETIC BASIS IN COMMON BEAN (PHASEOLUS VULGARIS L.)

A Dissertation in
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by
Magalhaes Amade Miguel

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The dissertation of Magalhaes A. Miguel was reviewed and approved* by the following:

Jonathan P. Lynch  
Professor of Plant Nutrition  
Dissertation Adviser  
Chair of Committee

Kathleen M. Brown  
Professor of Post-harvest Physiology

Roger T. Koide  
Professor of Horticultural Ecology

Barbara J. Christ  
Professor of Plant Pathology

Richard P. Marini  
Professor of Horticulture  
Head of the Department of Horticulture

* Signatures are on file in the Graduate School.
ABSTRACT

FUNCTIONAL ROLE AND SYNERGYSTIC EFFECT OF ROOT TRAITS FOR PHOSPHORUS ACQUISITION EFFICIENCY AND THEIR GENETIC BASIS IN COMMON BEAN (PHASEOLUS VULGARIS L.)

Magalhães A. Miguel
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The Pennsylvania State University
Jonathan P. Lynch, Thesis Advisor

The rapid increase of the global population, especially in the developing countries requires an increase in crop productivity to meet with current demands. However, in these countries several factors limit crop yields, especially low soil fertility and drought. In most of the countries in Latin America and Africa, more than half of the cultivated areas are affected by low phosphorus availability. Most of existing cultivars are not suitable for these unfavorable soils. The majority of the farmers in these regions cannot afford the application of chemical fertilizers to improve their soil fertility. Therefore, there is a need to identify root traits conferring phosphorus acquisition efficiency and to develop cultivars adapted to phosphorus stressed soils.

Root architectural phenes enhancing topsoil foraging are important for phosphorus acquisition. Here we describe the utility of a novel phene, basal root whorl number (BRWN), that has significant effects on topsoil foraging in common bean (Phaseolus vulgaris L.). Whorls are defined as distinct tiers of basal roots that emerge in a tetrarch fashion along the base of the hypocotyl. In this study, wild and cultivated bean taxa as well as a Recombinant Inbred Line (RIL) population were screened for BRWN and basal root number (BRN). A set of 6 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. Wild bean accessions tended to have one or two BRWN whereas cultivated accessions had BRWN reaching 4 and sometimes 5. 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 soils. Genotypes with greater BRWN had almost twice the shoot biomass, greater root length (90 cm vs. 50 cm length), and greater leaf area (1.7 m² vs. 0.89 m²) than related genotypes with less BRWN. In low phosphorus soil, shoot phosphorus content was strongly correlated with BRWN ($r^2 = 0.64$ in the greenhouse and $r^2 = 0.88$ in the field). Genotypes with three whorls had shallower root systems with a greater range of basal root growth angles (from 10 to 45 degrees from horizontal) than genotypes with two whorls (which ranged from 60 to 85 degrees from horizontal). Our 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.

In addition, we performed a quantitative trait loci (QTL) analysis for BRWN using recombinant inbred lines (RILs) developed from two populations. Basal Root Whorl Number (BRWN) is a root architectural trait in common bean that plays an important role in soil exploration and resource acquisition. BRWN varies from one to five among bean genotypes, and is an important determinant of basal root number (BRN), with each whorl typically forming four basal roots. The objective of this study was to perform a quantitative trait loci (QTL) analysis for BRWN and BRN using two populations of recombinant inbred lines (RILs) developed from the crosses DOR364 x G19833 and G2333 x G19839. Phenotypic data on the number of basal root whorls and number of basal roots was measured on seedlings 3 days after imbibition. QTL analysis for basal root whorl number and total basal root number was performed using composite interval mapping in these two populations using four phenotypic datasets. We found a total of 23 QTL associated with BRWN and BRN in the two populations. In the DOR364 x G19833 RIL population, we found 3 QTL in the first dataset with one QTL controlling 14.6% of the variation. For the fourth dataset, we found 7 QTL with one QTL controlling 23.8% of the variation in BRWN. For BRN, we detected 3 QTL in the 2005 dataset with one QTL controlling 13.7% of the variation. In the fourth dataset, we found 7 QTL on 5 linkage groups. One of the QTL on linkage group B7 controlled 25.9% of all the variation for BRN in that population. Variability in BRWN in the G2333 x G19839 RIL population was controlled by only one locus on linkage group B3. For basal root number
in the DOR364 x G19833 RIL population, we detected 4 QTL on B3, B6 and B7 in the first trial, and two QTL on B2 in the second trial. No QTL was found in the third trial. For the fourth dataset we found one QTL in linkage group B3 controlling 19.3% of the variation in BRWN. This proportion of variation explained by relatively few loci suggests that the potential for genetic manipulation of these traits via these locus is very good. As we have observed in the results of QTL analysis of phenotypic data from four different data sets over the years, QTL were detected in different parts of the genome. It appears that there are several regions which contain QTL or genes that can contribute to the development of basal roots.

We also tested the hypothesis of the existence of synergetic effect between root characteristics responsible for nutrient acquisition efficiency in Common bean. Multiple root traits affect phosphorus acquisition, including root hair length and density (RHLD), and basal root growth angle (BRGA). Shallow BRGA is an important trait for phosphorus acquisition efficiency by enhancing topsoil foraging, since in most soils, phosphorus is concentrated in the topsoil. Root hairs substantially increase phosphorus acquisition by expanding the soil volume subject to phosphorus depletion through diffusion. We hypothesized that shallow BRGA and long root hairs are synergetic for phosphorus acquisition, meaning their combined effect is greater than the sum of their individual effects. The purpose of this study was to evaluate this hypothesis by quantifying the effect of root hairs and basal root growth angle alone and in combination among closely related genotypes. We established a set of field experiments with Recombinant Inbred Lines (RILs) of common bean (Phaseolus vulgaris L.) grouped in 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. Results revealed substantial synergism between the two phenes. Long root hairs increased shoot biomass under phosphorus stress by 89.3% while shallow roots increased shoot biomass by 57.7%. Genotypes with both long root hairs and shallow roots had the greatest biomass accumulation, 298% greater than short-haired, deep-rooted phenotypes. Shoot biomass and phosphorus content of genotypes with long root hairs on deep roots and shoot biomass of genotypes with short root hairs on shallow roots did not differ, but were greater than those of genotypes with short root hairs on deep roots. We conclude that the
morphological phene of longer root hairs and the architectural phene of shallower basal root growth are synergetic for phosphorus acquisition.
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The reader of these lines is a bit fortunate than I, because he/she knows what happened with all this investment, and where I am…

Magalhães

‘Knowledge is Wealth’
- Amade Abacar (1912-1980).
Chapter 1

General Introduction

Low soil fertility is a type of a-biotic stress that is considered to be one of the major limiting factors for crop production throughout the world, especially in tropical ecosystems. Soils in tropical and sub-tropical ecosystems are old and highly weathered, and as result of this and constant cultivation, these soils show low content of such nutrients as N, P, K, Ca and Mg. In addition, these soils are acidic with very low pH, and are subject to excess of such metals as Al (specially Al$^{3+}$), Mn, Fe and other heavy metals causing problems of toxicity in plants. Nitrogen content has been depleted in these soils as a result of repeated cultivation, including slash and burn methods, and high rates of N leaching. The vast majority of producers in these regions are subsistence farmers and cannot afford to correct soil fertility problems through intensive phosphorus and nitrogen fertilizations or soil amendments because of the limited access to economic resources.

Phosphorus availability is generally low in volcanic soils (Andosols) with high allophane content, in highly weathered mineral soils (Oxisols, Ultisols, some Alfisols) characterized by Fe and Al oxide chemistry, and in coniferous forest soils (Spodosols), (Lynch and Beebe 1995). That means that phosphorus is commonly found bound to soil constituents that make it almost unavailable to plants. Yet these soils represent areas where most of the world’s vegetation and human population is supported.

Since economic difficulties related to soil fertility cannot be solved simply through application of chemical fertilizers and amendments, it is necessary to find a sustainable means to improve crop performance in these unfavorable soils. Genetic improvement for nutrient acquisition efficiency, especially phosphorus efficiency, in crops may be a viable alternative or complement to fertilization. It has been known for a long time that crop genotypes may differ substantially in adaptation to low phosphorus soils (Smith et al. 1994). Furthermore, studies have shown that a more diversified array of plant mechanisms have evolved to take up phosphorus under limiting conditions, including mycorrhizal symbioses, rhizosphere modification with organic acids, protons

Plant root characteristics are of primary importance in determining the ability of a plant to acquire nutrients and water. Certain plant species and genotypes within species are more efficient in extracting immobile phosphorus from the soil. This efficiency may be attributed to the changes in root system traits (Clark, 1991; Ma et al. 2001; Lynch 2005). These traits include root architecture, adventitious rooting, root biomass, root length, specific root length, root hairs and mycorrhizal associations (Lynch 2005). In addition, rhizosphere modification through secretion of carboxylates, protons and phosphatases are also known to play an important role in P acquisition efficiency (Lynch 2007).

Root architecture is defined as the spatial configuration of the root system over time, with the implication that the overall configuration has some functional significance (Lynch 1995). Root shallowness is important for P acquisition through topsoil foraging. Because topsoil is generally the soil stratum with greatest phosphorus bioavailability, adaptation to low soil phosphorus availability is associated with the variation in extent of topsoil foraging among genotypes of maize and common bean (Zhu et al. 2005; Ge et al. 2000). In common bean, topsoil foraging is strongly associated with phosphorus acquisition in low phosphorus tropical soils (Bonser et al. 1996; Liao et al. 2001; Lynch and Beebe 1995). In wheat, root length density in the upper soil layers was the most important trait associated with improved phosphorus absorption (Manske et al. 2000). Growth regulation is one of the adaptive mechanisms for P acquisition. A common response to phosphorus deficiency is an increase in root to shoot dry-weight ratio, resulting from a greater inhibition of shoot growth than root growth (Whiteaker et al. 1976; Lynch et al. 1991). Most species allocate more biomass to roots when inorganic P is limiting for their growth (Brouwer, 1963, 1983). Effects of inorganic phosphorus (P\textsubscript{i}) supply on biomass partitioning between roots and shoots are thought to involve a decreased production in and export of cytokinins from roots at a low P\textsubscript{i} supply, possibly associated with a decreased rate of uptake and metabolism of nitrogen (Kuiper et al. 1989). The increase in root biomass is usually associated with an increase in total root
length, which will significantly increase root surface area, enabling P stressed plants to absorb more phosphorus from the soil.

Genetic variation for phosphorus efficiency in bean has been demonstrated over 25 years ago (Whiteaker et al. 1976). Subsequent studies showed that such variation was heritable and was related to root traits. Lynch and co-workers have shown substantial variation in bean phosphorus efficiency, and it was found to be stable across soil environments in Latin America (Lynch and Beebe 1995). Analysis of the CIAT germplasm collection has identified several sources with outstanding phosphorus efficiency, ranging from 100 to 200% better than existent cultivars, such as Carioca (Yan et al. 1995). Physiological and agronomic analysis of these genotypes show that phosphorus efficiency in bean is related to efficient soil exploration at minimal root carbon expense, and that it can in part be attributed to root characteristics, including the presence of root hairs (Nielsen et al. 2001), shallow basal roots (Liao et al. 2001), or adventitious roots that enhance the volume of topsoil foraging (Ochoa et al. 2006). Species with greater root hair length and density, and specific root architecture patterns, exhibit increased phosphorus uptake (Nielsen et al. 1994; Bates et al. 1995). Another trait possibly related to P efficiency may be rhizosphere modification by root exudates (Yan et al. 2004). According to (Lambers et al. 2006), in addition to morphological traits, organic acids and phosphatases enhance inorganic phosphorus uptake under low P availability. Another root trait important for P acquisition efficiency is the presence of aerenchyma, which leads to a reduction of metabolic costs for root formation and maintenance during respiration (Lynch 2007). We need a better understanding of all these traits in order to guide the development of phosphorus efficient crops, important for developing countries, with limited access to inorganic fertilizers.

**Importance of Beans in developing countries**

Beans are the most important grain legumes for direct human consumption in the world. Total production exceeds 23 million metric tonnes (MT) of which 7 million MT are produced in Latin America and Africa (Broughton et al. 2003).
Common bean (*Phaseolus vulgaris* L.) is among the most important crops in Latin America and Southern Africa. In these regions, common bean has nutritional, ecological and economic values. Common bean is a principal source of protein and micronutrients, since the majority of rural population cannot afford to get protein from animal sources. Common bean plays an important role in many agronomic systems, helping to improve soil fertility mainly through incorporation of nitrogen from the atmosphere by biological nitrogen fixation. Common bean has economic value because in most of the production systems of small scale farmers in southern Africa it serves as a cash crop, with better market values than most cereals (maize and sorghum) and cassava, which are staple food crops but yield low incomes as cash crops. Farmers usually reserve a significant part of their production of common bean to sell at local market and use the income to pay for such basic needs as education of their children, medicine and household supplies such as Kerosene for illumination, soap for laundry, and other products that they cannot get from the farm like sugar, cooking oil and salt. Therefore, increased bean production in these areas would have a direct impact on a) population’s nutritional status, b) soil nutrient management and c) household economic welfare of rural farmers and their communities.

One way to address the question of how to increase bean production in such infertile soils is to enhance our understanding of the characteristics and mechanisms associated with nutrient efficiency. Phosphorus efficient beans will have higher yields compared to currently used P-inefficient beans when grown in soils with low phosphorus availability. An increase in bean production and productivity in these unfertile soils without significant application of P-fertilizer will improve social welfare of the majority of rural populations in Sub-Saharan Africa and Latin America.

Improvement in crop productivity in low P ecosystems can be achieved by understanding root traits and physiological mechanisms associated with nutrient acquisition efficiency.

**Root traits and phosphorus acquisition efficiency in common bean**

There is genotypic variation for a number of root traits responsible for nutrient
acquisition efficiency in common bean. An efficient common bean genotype can exhibit one or several traits conferring nutrient acquisition efficiency. Traits that are associated with enhanced topsoil foraging in common bean include shallower growth of basal roots, enhanced adventitious rooting, and greater dispersion of lateral roots (Lynch 2005).

There is genotypic variation for a number of root traits responsible for nutrient acquisition efficiency in common bean. These traits include basal root whorl number, basal root growth angle, adventitious rooting, aerenchyma formation, and the ability to form long and dense root hairs in low phosphorus environments, among others. Most of these traits can be assessed in the lab, greenhouse or field. Ochoa et al., 2006 found that adventitious roots can significantly increase P uptake in common bean grown both in greenhouse and in the field. Shallow rooted common bean genotypes do better compared to deep-rooted genotypes when grown in low P soils, since phosphorus concentration along soil profile decreases significantly with depth. (Bates and Lynch 2001) have shown that root hairs play an important role for P acquisition. (Zhu et al. 2005) also found root hairs to be beneficial for P acquisition in maize. Root hairs can be beneficial even in the presence of mycorrhizal colonization in common bean grown in the greenhouse (Miguel, 2004). By replacing living cells with air, RCA significantly reduces both the respiratory and nutrient requirements of root tissue, permitting greater root growth and nutrient acquisition for a given metabolic investment (Lynch 2007). Recently, we observed that a new trait seems to have a significant contribution for nutrient acquisition efficiency in plants grown under low soil fertility.

1. **Basal root whorl number: a novel phene for increased phosphorus acquisition in common bean (Phaseolus vulgaris L.)**

   Whorls are defined as distinct tiers of basal roots that emerge in a tetrarch fashion along the base of hypocotyls. The whorl closest to the shoot produces the shallowest roots, and lower whorls produce deeper roots. Genotypes vary in both growth angles of basal roots and the number of basal whorls (Bonser et al. 1996; Basu et al. 2007). This variation can be observed in 4-day old seedlings grown in germination paper and placed in the growth chamber. Previous studies have shown genetic variation for basal root whorl number ranging from 1 to 4 and sometimes up to 5 whorls, and consequently
variation in total number of basal roots (Widrig, 2005). This variation in number of root whorls has been correlated to phosphorus uptake efficiency in bean (unpublished data). Root architecture, which can determine plant’s ability to acquire nutrients, can be greatly affected by these patterns of root system formation. The concept of root whorl is relatively new, and basal root whorl number might have a significant influence in root distribution through soil profile, which in turn can affect the ability of the plant to acquire nutrients, especially when they are scarce or not evenly distributed.

Basal root whorl number (BRWN) and Basal root Growth Angle (BRGA), do not appear to be influenced by environment. (Basu et al. 2007) found that genotype had a much greater effect on BRGA than phosphorus treatment. In this study, the authors found consistent variation in BRGA among bean genotypes grown both in high and low phosphorus availability. However, (Bonser et al. 1996) found that in some genotypes BRGA was influenced by P availability. This enables detection of Quantitative Trait Loci (QTL) controlling this trait.

### 2. Quantitative Trait Loci (QTL) analysis of basal root whorl number in Common Bean (*Phaseolus vulgaris* L.)

In the absence of evidence implicating a specific gene (i.e., a candidate gene) that may contribute to a quantitative trait, researchers can employ several strategies to determine the role of an individual DNA sequence among all the other genetic and non-genetic influences on the trait.

The dissection of a quantitative trait into its discrete genetic determinants, namely the QTL, is achieved through the combined analysis of phenotypic and molecular data collected from experimental or natural populations segregating for the trait(s) of interest (Lee 1995). More commonly, QTL for basal root whorl number are identified through the evaluation of mapping populations of families in the F3 generation or at higher levels of inbreeding (e.g., F7 or F8 families), particularly in autogamous species which, compared to allogamous species, suffer less from inbreeding depression. QTL analysis is useful for difficult to measure traits, such as root traits, since they are more easily employed by plant breeders for selection. QTL analysis has been performed for such root traits as root
hairs and acid exudation traits, and their relationship to phosphorus uptake in common bean (Yan et al. 2004). QTL analysis has been performed for seminal root traits in maize seedlings grown under differential phosphorus levels (Zhu et al. 2005), QTL analysis has been performed for adventitious formation in common bean (Ochoa et al., 2006), grown in low P both in the greenhouse and in the field.

Quantitative Trait Loci analysis for root traits seems promising and recent studies have suggested that QTL analysis can be of a particular importance to plant breeders for selection for such root traits difficult to measure using conventional breeding tools alone.

3. Phene synergism between root hairs and basal root growth angle for phosphorus acquisition in common bean

a) Root hairs

Root hairs play an important role for uptake of nutrients that are less mobile in the soils, such as phosphorus. Root hairs are believed to enhance P acquisition by increasing soil volume explored by the plant (Clarkson 1985; Peterson and Farquhar 1996). Early work with autoradiography demonstrated that 32P-phosphate is depleted around roots and the volume of the depletion zone is affected by the length of root hairs (Bhat and Nye 1973). Studies with many species showed a significant correlation between radius, density and length of root hairs and P content in plants, indicating a close relationship between root hairs and P uptake from the soils (e.g., Foehse and Jungk 1983; Herrmann et al. 1995). In barley and wheat, genotypic variation in root-hair length could significantly affect P uptake from the soil (Gahoonia et al. 1997; Gahoonia and Nielsen 1997).

Root hair formation is dependent on internal and external factors. Many plant species exhibit increased extension of root hairs in response to phosphorus deficiency (Bates and Lynch 2000). There is a genetic variation of root hairs length in common bean. Long root hair genotypes are more efficient in acquiring less mobile nutrients in the soil than genotypes with short root hairs. Root hairs usually are found 1-2 cm from the root tip, and all the hairs have a determined life span, after which their number (density) is greatly reduced. Root hair density is also important for P acquisition efficiency. In
some plant species, like Arabidopsis, the increase in root hair density in the conditions of low phosphorus supply is thought to be partly due to anatomical changes leading to an increased number of trichobast files (Ma et al. 2001).

Yan et al. 2004 studied the relationship between root hair growth, acid exudation and phosphorus (P) uptake in recombinant inbred line (RIL) population derived from the cross of two contrasting common bean genotypes, and found significant genotypic variability for root hairs. Root hair growth was regulated by P availability, and P deficiency significantly increased root hair density and length in some genotypes. Studies with Arabidopsis thaliana have shown that low phosphorus availability increases root hair length, accompanied by increased root hair density (Bates et al. 1995; Bates and Lynch 2000). The increase in root hair length and density contributes to nutrient acquisition efficiency during plant growth in low soil fertility ecosystems.

A strong correlation was found between root hair length and root hair density among genotypes of common bean (Miguel, 2004). This suggests that low phosphorus availability in the soil that leads to root hair elongation seems also to affect root hair density, enhancing therefore the contribution of root hairs, possibly through synergistic relationship of various root traits for phosphorus uptake.

Thus, a better understanding of genetic variability of plant materials on root hair traits opens a possibility of crop yield increase through genetic improvement.

b) Basal Root Growth Angle (BRGA)

Basal root growth angle is a major determinant of whether a genotype has shallow or deep roots, which in turn can affect the ability of the plant to acquire phosphorus. Since phosphorus is more concentrated in the top layer of soil profile, having shallow roots is (small BRGA) regarded to be beneficial for phosphorus acquisition, especially when plants are grown with low phosphorus supply. In an experiment conducted in the field, shallow rooted recombinant inbred lines of common bean responded dynamically to phosphorus availability, which permitted greater root exploitation of topsoil, and greater biomass and phosphorus accumulation (Liao et al. 2004).
Previous studies have shown that changes in root architecture can have a significant impact on a plant’s ability to acquire certain resources from the soil. For example, deep-rooted bean genotypes are regarded to be efficient in water acquisition under drought, while shallow rooted genotypes are regarded to be suited for phosphorus acquisition in low phosphorus, stratified soils (Ho et al. 2004).

Basal Root Growth Angle (BRGA) ranges from nearly 0° (horizontal) to 90° (vertical). Some genotypes have consistent BRGA under high or low phosphorus, whereas other genotypes are plastic, i.e. they respond to soil stress by changing their BRGA to acquire more of the limiting resource. Plasticity is regarded as a good trait since it would permit an optimization of architectural trade-offs in root functions (Ho et al. 2004). Variation in BRGA plasticity also exists for drought (Ho et al. 2004). The potential utility of this trait has not been extensively investigated, but it is possible that BRGA plasticity permits plants to optimize their root deployment depending on the severity and type of stress they experience (Ho et al. 2004; Ho 2004; Ho et al. 2005). Given the substantial genotypic variability for BRGA that has been observed (Bonser et al. 1996) and (Basu et al. 2007), it should be possible to use this trait as a selection criterion in bean breeding. Researchers have been attempting to evaluate the utility of this trait under drought and low phosphorus and identify QTL associated with it. The adaptive value of root growth plasticity in response to drought has never been rigorously evaluated in any crop but this trait seems very exciting.

c) Synergism between root traits

Several root traits have been identified as responsible for phosphorus acquisition efficiency. For example, root hairs are reported to significantly increase phosphorus acquisition in Arabidopsis (Ma et al. 2001), maize (Zhu et al. 2005) and common bean (Yan et al. 2004; Miguel, 2004). Genotypes with small BRGA (shallow-rooted genotypes) have greater P accumulation compared to deep-rooted genotypes (Liao et al. 2004). It is possible that shallow rooted genotypes with long root hairs acquire more phosphorus than the sum of P acquisition caused by the presence of long root hairs and greater BRGA occurring separately. (Ma et al. 2001) found synergistic effect between root hair length and density in Arabidopsis. In our study, we hypothesize the existence of
synergistic effect among root traits for phosphorus acquisition. To what extend these two traits have a synergistic effect on P uptake has not been investigated. Because of these differences in strategy employed by each of these root traits for P acquisition efficiency, a positive synergistic effect may exist, and it is possible to determine and quantify.

4. Objectives of the study

This study of root traits related to phosphorus acquisition efficiency in bean has the following objectives:

a) Evaluate of the utility of a novel trait, basal root whorl number (BRWN), in common bean for phosphorus acquisition under low phosphorus availability. We used common bean genotypes contrasting for this trait in studies conducted both in greenhouse and in the field.

b) Perform Quantitative Trait Locus Analysis for BRWN in two RIL populations of common bean. Identify the number and location of QTL that might be associated with this trait.

c) Determine and quantify synergism between root hairs and basal root growth angle, two root traits contributing to phosphorus acquisition efficiency in soils with low phosphorus availability.
References


Chapter 2

Basal root whorl number: a novel phene for increased phosphorus acquisition in common bean (*Phaseolus vulgaris* L.)

Miguel, M.A.¹, A. Widrig¹, R. Vieira¹², K. M. Brown¹, and J. P. Lynch¹³

¹Department of Plant Science, The Pennsylvania State University, University Park, PA 16802, USA.
²Empresa de Pesquisa Agropecuária de Minas Gerais, Caixa Postal 216, CEP 36571-000 Viçosa, MG, Brazil.
³Corresponding author. Email: JPL4@psu.edu

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Abstract

Root architectural phenes enhancing topsoil foraging are important for phosphorus acquisition. Here we describe the utility of a novel phene, basal root whorl number (BRWN), that has significant effects on topsoil foraging in common bean (*Phaseolus vulgaris* L.). Whorls are defined as distinct tiers of basal roots that emerge in a tetrarch fashion along the base of the hypocotyl. In this study, wild and cultivated bean taxa as well as a Recombinant Inbred Line (RIL) population were screened for BRWN and basal root number (BRN). A set of 6 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. Wild bean accessions tended to have one or two BRWN whereas cultivated accessions had BRWN reaching 4 and sometimes 5. 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 soils. Genotypes with greater BRWN had almost twice the shoot biomass, greater root length (90 cm vs. 50 cm length), and greater leaf area (1.7 m² vs. 0.89 m²) than related genotypes with less BRWN. In low phosphorus soil, shoot phosphorus content was strongly correlated with BRWN ($r^2=0.64$ in the greenhouse and $r^2=0.88$ in
the field). Genotypes with three whorls had shallower root systems with a greater range of basal root growth angles (from 10 to 45 degrees from horizontal) than genotypes with two whorls (which ranged from 60 to 85 degrees from horizontal). Our 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.

Introduction

Low phosphorus availability is a primary constraint for crop productivity in most tropical soils of Africa, Latin America and Asia (Lynch 2007; Vance et al. 2003). For example 85% of all common bean (Phaseolus vulgaris L.) 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 spatial variation in 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 (Hinsinger 2011; Ryan et al. 2001), 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 and Brown 2001).

Plants under phosphorus stress cannot simply grow more roots throughout the soil profile without slowing plant growth by diverting resources from photosynthesis. 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 effectively remobilize phosphorus 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, 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, adventitious (shoot-borne) roots emerging from the subterranean hypocotyl, and lateral roots developing from each of the other root classes (Zobel 1986; Lynch and van Beem 1993). Recently we have observed that 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’. Typically four roots emerge from each whorl. Phenotypic profiling of Phaseolus taxa revealed significant variation in basal root whorl number (BRWN) as well as the number of basal roots (BRN; Widrig, 2005). We hypothesize that genotypes with greater BRWN will have more dispersed basal roots, with those coming from the uppermost whorls being more shallow, increasing topsoil foraging and phosphorus acquisition (Widrig, 2005). Root shallowness and dispersion enhance root proliferation in phosphorus-rich topsoil and reduce competition among roots of the same plant (Ge et al. 2000). In addition genotypes with more basal roots may display a greater range of growth angles, including shallow roots as well as deeper roots, which would be important for water acquisition from drying soil. In this study we attempt to determine the functional role of basal root whorl number for phosphorus acquisition.
Materials and Methods

Greenhouse Experiment

Plant Materials

For the greenhouse experiment, six contrasting genotypes from Brazil were used in the experiment. The genotypes included: Genotypes 13, 73, and 76 with two basal root whorls in average and genotypes 43, 51 and 62, with three basal root whorls in average.

Information on other root traits on the RILs from which we selected the 6 phenotypes used in this experiment were determined prior the study, and 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.

Initial screening

We initially screened in the laboratory a number of wild and cultivated bean taxa, and RILs of two populations of common bean for BRWN. Seeds were surface sterilized with 0.5% NaOCl for one minute before being scarified and placed onto low phosphorus brown germination paper (Anchor Paper, St. Paul, MN, USA.) saturated with 0.5 mM CaSO4. Five seeds of each genotype were placed 2 cm from the top of a 20 cm long piece of germination paper and rolled into a moderately tight ‘cigar roll’ configuration and placed in a 1 L beaker with 100 mL of 0.5 mM CaSO4. The beakers were filled with 12 to 13 rolls before being wrapped with cellophane, punctured with holes to allow aeration. The beakers were then placed into a germination chamber for 4 to 5 days at 28 °C. The basal root whorl number and basal root number per seedling were then recorded.

Genotype screening for greenhouse experiment

A RIL population was evaluated in the laboratory using the ‘roll-up’ method
described above. Seeds were sterilized in 0.5% NaOCl for 1 minute and washed twice in de-ionized H2O before being rolled in brown germination paper and placed in 47.5 cm x 35 cm x 25 cm (length x width x height) plastic containers filled with 50 ml of 0.5 mM CaSO4. The containers were placed in a germination chamber at 28 ± 1 °C for three days in darkness. On the fourth day, the seedlings were visually evaluated for basal root whorl number and basal root number. From this screening, six contrasting RILs were selected for use in the greenhouse experiment. Seeds of selected lines were surface-sterilized for 1 to 2 min in 10% (v/v) NaOCl, thoroughly rinsed with de-ionized water, mechanically scarified, and germinated in rolls of brown germination paper (Anchor Paper Co., St. Paul, MN, USA), and then placed upright in 500-ml beakers containing 200 ml of 0.5 mM CaSO4. Then seedlings were transplanted to 20-liter pots in the greenhouse.

Growth Media

A mixture of 50% commercial grade medium size yellow sand, 40% D3 coarse vermiculite (Whittemore Co., Inc., Lawrence, MA) 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-liter 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 4µM KH2PO4 for low phosphorus treatments and 500 µM KH2PO4 for high phosphorus treatments. The other nutrients were: 1.5 mM KNO3, 1.2 mM Ca(NO3)2, 0.4 mM NH4NO3, 0.025 mM MgCl2, 0.5 mM MgSO4, 0.3 mM K2SO4, 0.3 mM (NH4)2SO4, 5 µM Fe-EDTA, 1.5 µM MnSO4, 1.5 µM ZnSO4, 0.5 µM CuSO4, 0.15 µM (NH4)6Mo7O24, and 0.5 µM Na2B4O7. 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, when plants were smaller, and starting at four 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 P (4µM KH₂PO₄) and high P (500 µM KH₂PO₄). Each treatment had 4 replications of 3 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 to August 2006. The average temperature was 26°C, mid-day photosynthetic active radiation (PAR) averaged 800 to 1000 µmol photons m⁻² s⁻¹, and the average humidity was 60%. Natural light was supplemented from 800 to 2000 h with 110 µmol photons m⁻² s⁻¹ from 400W metal halide bulbs (Energy Technics, York, PA, USA).

Data Collection and Analysis

Data collected in the greenhouse experiment included total shoot dry weight, tissue phosphorus content, total root length, basal root whorl number and basal root number. Plant samples were collected at 14, 21 and 28 days 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 was 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). Basal root whorl number and basal root number 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 Test for comparisons between means, and regression analysis was performed using Minitab (Minitab Inc., State College, PA, USA).
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, GG 37 and GG 80), and three whorls (G19839, GG 41 and GG 48). These genotypes were selected after screening the entire population of 87 recombinant inbred lines from this cross in roll-ups in the laboratory.

Experimental Design

A completely randomized design was used in the field experiment. Treatments were established under low and high phosphorus availability. Low phosphorus had 6 ppm available P (Olsen), while high phosphorus treatments had 19 ppm available P (Olsen). The fertilizers were applied in the field in the corresponding amounts by hand prior the establishment of the experiment in the field. Each treatment had 4 replications of each of 3 genotypes per BRWN phenotype (2 vs. 3 whorls). Each plot contained three rows two meters in length with 60 cm between rows. Twenty-one seeds were planted in each row, with a spacing of 10 centimeters within the row (60 cm x 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 m.a.s.l.). The soil type at the research site is an Ustox with low pH (4.5 to 5.5). Three months before planting, the soil was limed (CaCO₃) 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 P for fertilized plots applied at the rate of 100 kg per ha. 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 P in the top 15 cm with a rapid decline below 15 cm depth both in low and medium phosphorus plots. Weed control was performed manually. Pesticides were applied as needed.

Data collection and analysis

Collected data included shoot dry weight, total root length, total leaf surface area, basal root whorl number, basal root number, and total phosphorus content. Plant samples were collected in three harvests at 14, 21, and 28 days after planting. Shoot biomass was determined from samples dried at 60 °C for 5 days. Tissue phosphorus content was determined spectrophotometrically (Murphy and Riley, 1962). During shoot sampling, leaf discs (6.6 cm²) were collected from five fully expanded leaves. The ratio of dry weight to area of these disks was used to estimate total shoot leaf area from total leaf dry weight. At plant sampling, roots crowns were excavated and placed in a 20-liter container with soap for washing. Detergent was added to the water used to wash the roots 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 obtained from root crowns were washed and rinsed in clean tap water and placed in the vials with 25% ethanol solution for preservation. Roots from root crowns were scanned on an EPSON Perfection V700 PHOTO scanner from ICE digital technologies (Epson UK Ltd. Westside, London Road Hemel Hempstead Herts). The images were analyzed for total root length, and root length by root diameter class, using WinRhizo Pro. In order to evaluate root distribution with soil depth, we took cores in the field at 28 DAP. Root
coring consisted of extracting soil samples by hammering a 5-cm diameter metal cylinder vertically into the soil between two bean plants spaced at 10 cm apart within the planting row. Cores were separated into 0-15 cm and 15-30 cm soil depths. Root fragments were recovered from each of these soil sections and analyzed using WinRhizo Pro (Regent Instruments, Quebec, CA) for root length determination. Although genotypes were selected based on their BRWN, plants harvested at 14 DAP 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. Analysis of variance (ANOVA), Tukey test for comparisons between means, and regression analysis were conducted using Minitab (State College, PA, USA).

Ranges in angle were obtained from 24 plants (3 genotypes in 4 replications under low P treatments in the initial screening. Genotypes were considered shallow-rooted if the average angle of all the whorls were less than 45 degrees, deep rooted if the average angle of all whorls were more than 45 degrees from horizontal.

Results

Initial screening

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. The number of basal roots was determined by the number of whorls, with approximately four basal roots per whorl (Figure 2.1). Wild accessions had 1 to 2 whorls and 4 to 8 basal roots while cultivated taxa had 2 to 4 basal root whorls and 8 to 16 basal roots (Figure 2.1). Six out of 35 CIAT parents and elite lines screened for basal root whorl number and basal root number had three whorls and 12 basal roots, while the rest of the lines had an average of two basal root whorls and 8 basal roots (Table 2.1). No correlation (R² = 0.069) was found between BRWN and seed weight in 63 wild P. vulgaris accessions (Figure 2.2). Although BRWN varied from 1 to 4, most genotypes had two whorls (Table 2.2). 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 2.3). Significant genotypic variation in basal root whorl number was observed among RILs from the DOR364 x G19833 population. BRWN was closely correlated with the BRN. Genotypes with greater BRWN had a greater range of growth angles of basal roots than genotypes with fewer whorls.

Greenhouse Experiment

Under low phosphorus availability, there was a strong positive correlation ($r^2=0.64$) between BRWN and shoot dry weight (Figure 2.3) and shoot P content (Figure 2.4a). No significant correlation was observed between BRWN and shoot phosphorus content in plants grown under high phosphorus availability (Figure 2.4b). There was no correlation between adventitious roots and basal root whorl number and basal root number, regardless of phosphorus treatment (data not shown). Genotypes with less BRWN had greater percent reduction in phosphorus content compared to genotypes with greater BRWN, when grown under low phosphorus availability (Figure 2.5). Low phosphorus reduced root length by 60.1% in 2-whorl genotypes, versus 44.5% in 3-whorl genotypes (Table 2.4).

Field Experiment

BRWN and BRN phenotypes in the field were consistent with previous screening data. Genotypes G2333, GG37 and G80 had 2 whorls and 8 basal roots while genotypes G19839, GG41 and GG48 had 3 whorls and 12 basal roots on average (Figure 2.6 for BRWN and Figure 2.7 for BRN). Under low phosphorus, 3-whorl genotypes had greater shoot dry weight than 2-whorl genotypes (Figures 2.8, 2.9), as well as greater leaf area (Figure 2.10) and greater phosphorus content (Figure 2.11). BRWN affected phosphorus content in both low P and medium P treatments (Table 2.6).

Genotypes were also evaluated for root hair length. All genotypes had greater root hair length under low phosphorus availability compared to medium phosphorus availability but BRWN did not affect root hair length (Table 2.6). However, for
phenotype GG 48, root hair length did not vary with P treatment, and it was significantly less compared to other genotypes both under low P (Figure 2.12).

We collected soil cores at two depths in the field at 28 DAP to analyze root length distribution with depth. Genotypes with 3 whorls had more roots in the top 15 cm of soil than genotypes with 2 whorls, while genotypes with 2 whorls had greater root length in the 15-30 cm segments (Figure 2.13A and 2.13B). Total root length from soil cores (0-30 cm depth), was greater in genotypes with 3 whorls compared to genotypes with 2 whorls. Data from the scan of root crown, although not representing the entire root system, showed genotypes with three whorls that had greater root length compared to genotypes with two whorls both under low phosphorus and medium phosphorus availability (Figure 2.14). Differences in the number of adventitious roots were observed among genotypes, but they were not influenced by phosphorus availability (Figure 2.15). In addition, variation in adventitious rooting was not related to variation in BRWN among phenotypes, which shows that BRWN is independent from adventitious rooting, and thus, with no compensative effect for phenotypes with few basal root whorls.

**Discussion**

Our results are consistent with the hypothesis that greater BRWN is a positive adaptation to low soil phosphorus availability. Under non-limiting P availability, the presence of greater BRWN does not make significant difference in plant performance. Under low phosphorus availability in the field and in controlled conditions in the greenhouse, bean genotypes with 3 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 P acquisition is 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 P-limited environments since phosphorus availability in most soils is greatest in surface horizons (Lynch and Brown 2001). 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 being exploited by the plant, as shown by the greater range of basal root growth angles in genotypes with greater BRWN. We did
not observe compensatory reductions in adventitious rooting in high BRWN phenotypes. BRWN therefore appears promising as a phene for developing genotypes of bean and possibly other dicot crops with better productivity in low P soils.

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 less basal roots compared to cultivated accessions (Widrig, 2005). Greater BRWN in cultivated accessions may reflect plant adaptation to the less favorable environments that these accessions experienced with domestication, where crops where grown under continuous cultivation in soils subject to degradation, in soils beyond their initial range of adaptation.

Genotypes with 3 whorls may have a greater vertical range of soil exploitation compared to genotypes with 2 whorls. 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 P availability.

It is important to note that in the field study we did not have a truly high phosphorus treatment, but rather medium phosphorus- i.e. both P treatments were suboptimal 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 tradeoffs by diverting internal resources from competing uses such as axial elongation or branching, or shoot growth and reproduction. Such tradeoffs between basal roots and adventitious roots have been demonstrated in bean (Walk et al., 2006). In this study, we observed no correlation of adventitious rooting with variation in BRWN. However, we observed an increase in the number of adventitious roots in the low phosphorus treatment in all genotypes regardless of BRWN. This confirms earlier observations that low phosphorus availability increases adventitious rooting in common bean (Miller et al. 2003, Ochoa et al., 2006). Tradeoffs 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 tradeoffs
should be understood for informed deployment of this trait in plant breeding. Increased basal root whorl number may also be beneficial for resistance to biotic stresses. A phenotype with greater BRWN has also greater BRN, and assuming that there would be a feedback between BRN and photosynthesis, it is possible that high BRN could provide 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 herbivore or disease.

Basal root whorl number appears to be an important trait for P acquisition in plants growing under low phosphorus availability. The process of germplasm screening for BRWN is relatively easy and straightforward. Seedlings can be nondestructively phenotyped for BRWN and BRN 3 days after germination using simple techniques in the lab. However, BRWN as a trait is limited to those plant species with basal roots such as common bean and cowpea. In other species such as soybean, the concept of BRWN, as a defined root trait, does not exist, since the soybean root system does not have distinct basal roots, with all lateral and subsequent roots emerge from the primary root or other lateral roots. For these species different root traits are present, like the case of the presence of nodal root number in maize, which are also important for phosphorus acquisition when growth in low phosphorus ecosystems.
References


Figures

Figure 2.1. Variation of basal root whorl number and basal root number in 246 wild and cultivated common bean species. The number of basal roots is determined by the number of whorls, and there are about four basal roots per whorl. Wild *Phaseolus* species have fewer basal roots and whorls compared to their cultivated relatives.
Figure 2.2. Correlation between Basal Root Whorl Number and Seed Weight among wild *P. vulgaris* L. Each point is the average seed weight of 5 seeds corresponding to 5 treatments.

\[ y = 0.0309x + 0.0074 \]

\[ R^2 = 0.06991 \]
Figure 2.3. Correlation between shoot dry weight and basal root whorl number in six genotypes of common bean grown under low phosphorus availability in greenhouse conditions. A strong positive correlation ($r^2=0.641$) was observed at 28 DAP in the greenhouse. Each point is average of four replicates.
Figure 2.4. Correlation between shoot phosphorus content and basal root whorl number in six contrasting genotypes grown under low (A) and medium (B) phosphorus availability in the greenhouse. Each point is average of four replicates. Significant correlation was found under low P ($R^2 = 0.6423$), while under high P the correlation was not significant ($R^2 = 0.18326$).
Figure 2.5. Percentage reduction of shoot phosphorus in plants grown in the greenhouse under low phosphorus compared to medium phosphorus in six genotypes contrasting for basal root whorl number. Plants were harvested at 28 DAP. Each root category had 3 genotypes with four replicates.
Figure 2.6. Variation of basal root whorl number among six genotypes grown under low and high phosphorus availability in the field and evaluated at 14 DAP. Genotypes G19839, GG41 and GG48 were classified as having 3 basal root whorls and genotypes G2333, GG37 and GG80 were classified as having 2 basal root whorls. Each bar represents four replicates. Genotypes G2333 and G19839 showed consistent values in all four replicates. Therefore, standard error for these genotypes is zero.
Figure 2.7. Variation in basal root number (BRN) among six genotypes of common bean contrasting for basal root whorl number (BRWN). Genotypes with 3 basal root whorls had greater total basal roots compared to genotypes with 2 basal root whorls. Each mean was obtained from four replicates.
**Figure 2.8.** Shoot dry weight of six genotypes contrasting for basal root whorl number (BRWN), grown under low phosphorus (5 ppm) and medium phosphorus (19 ppm) in the field. Plant samples were collected at 28 DAP planting. Each mean was obtained from four replicates. Under low P treatment, genotypes with 3 whorls had statistically more shoot dry weight compared to 2 whorled genotypes (see Table 2. 7 for statistics).
Figure 2.9. Shoot dry weight of genotypes with 2 and 3 basal root whorls (BRWN), grown under low P (5 ppm) and medium P (19 ppm) in the field. Plant samples were harvested at 28 DAP. Harvests at 14 and 21 DAP did not show significant differences genotypes. Under low phosphorus, statistical significant differences were observed between the two root categories. Root categories did not differ in medium phosphorus treatments. Each mean was obtained from four replicates.
Figure 2.10. Leaf area of genotypes contrasting for basal root whorl number (BRWN) grown under low P (5 ppm) and medium P (19 ppm) in the field. Statistically significant differences were observed among genotypes grown under medium phosphorus and under low phosphorus availability. Each bar represents 4 replicates. Genotypes with 3 whorls showed greater leaf area compared to genotypes with 2 whorls, when grown with low phosphorus. Under low P treatment, genotypes with 3 whorls had statistically more leaf area compared to 2 whorled genotypes (see Table 2.8 for statistics).
**Figure 2.11.** Phosphorus content among six genotypes contrasting for basal root whorl number (BRWN), grown under low phosphorus (5 ppm) and medium phosphorus (19 ppm) in the field. Statistically significant differences for phosphorus content were observed among genotypes grown under both medium and under low phosphorus availability. In low phosphorus treatments, statistical analysis showed that genotypes with 3 whorls showed significantly greater phosphorus content in plant tissue compared to genotypes with 2 whorls. Each bar represents 4 replicates.
**Figure 2.12.** Root hair length among six genotypes of common bean contrasting for basal root whorl number. Genotypes showed greater root hair length under low phosphorus availability compared to high phosphorus availability regardless of BRWN (See Table 2.12 for ANOVA and Fisher test for low phosphorus treatments). Each mean was obtained from four replicates.
Figure 2.13. Root length recovered from soil cores at 0-15 cm (A) and 15-30 cm depth of soil profile (B). Genotypes with 3 whorls showed greater root length compared to genotypes with 2 basal root whorls, both under low and high phosphorus availability. Root length (in centimeters) is per core volume at each depth of soil profile (See Tables 2.13 and 2.14 for ANOVA). Each mean was obtained from four replicates.
**Figure 2.14.** Variation in root length among six genotypes of common bean contrasting for basal root whorl number (BRWN). Total root length was measured from excavated root crowns of 3 plants per each of four replication at 28 DAP grown in the field. Root length (in meters) is a sum of root length per core volume from 0-15 cm and from 15-30 cm soil profile (See Table 2.15 for ANOVA). Each bar is the mean of four replicates.
**Figure 2.15.** Adventitious roots in six common bean genotypes contrasting in BRWN. Plant samples were taken at 28 DAP in a field experiment. Each bar is the mean of four replicates.
Table 2.1. Screening of CIAT Parents and Elite Lines for basal root whorl number and basal root number. Average whorl and basal root number are based on the screening of 5 replicates (5 seeds) per genotype.

<table>
<thead>
<tr>
<th>Genotype/ RIL</th>
<th>Average number of whorls</th>
<th>Average number of roots</th>
<th>Standard deviation (number of roots)</th>
<th>Average seed weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G4494</td>
<td>3.20</td>
<td>12.00</td>
<td>2.50</td>
<td>0.58</td>
</tr>
<tr>
<td>CAL125</td>
<td>3.00</td>
<td>12.00</td>
<td>0.00</td>
<td>0.4</td>
</tr>
<tr>
<td>G14655</td>
<td>3.00</td>
<td>11.20</td>
<td>0.79</td>
<td>0.49</td>
</tr>
<tr>
<td>CERINZA</td>
<td>3.00</td>
<td>10.00</td>
<td>0.71</td>
<td>0.52</td>
</tr>
<tr>
<td>CAL149</td>
<td>3.00</td>
<td>10.20</td>
<td>0.84</td>
<td>0.53</td>
</tr>
<tr>
<td>ZPV292</td>
<td>3.00</td>
<td>9.60</td>
<td>0.89</td>
<td>0.36</td>
</tr>
<tr>
<td>TLP19</td>
<td>2.80</td>
<td>10.40</td>
<td>1.52</td>
<td>0.24</td>
</tr>
<tr>
<td>G19842</td>
<td>2.60</td>
<td>9.80</td>
<td>1.79</td>
<td>0.42</td>
</tr>
<tr>
<td>CRF61</td>
<td>2.60</td>
<td>9.20</td>
<td>1.30</td>
<td>0.24</td>
</tr>
<tr>
<td>MAR*1</td>
<td>2.60</td>
<td>9.00</td>
<td>1.00</td>
<td>0.25</td>
</tr>
<tr>
<td>CARIOCA</td>
<td>2.40</td>
<td>8.80</td>
<td>1.10</td>
<td>0.16</td>
</tr>
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<td>AND696</td>
<td>2.20</td>
<td>8.60</td>
<td>1.34</td>
<td>0.45</td>
</tr>
<tr>
<td>VAX1</td>
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<td>8.40</td>
<td>0.89</td>
<td>0.22</td>
</tr>
<tr>
<td>BF29</td>
<td>2.00</td>
<td>8.00</td>
<td>0.00</td>
<td>0.23</td>
</tr>
<tr>
<td>BF19</td>
<td>2.00</td>
<td>8.00</td>
<td>0.00</td>
<td>0.24</td>
</tr>
<tr>
<td>DICTA17</td>
<td>2.00</td>
<td>8.00</td>
<td>0.00</td>
<td>0.32</td>
</tr>
<tr>
<td>BF54</td>
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<td>8.00</td>
<td>0.00</td>
<td>0.27</td>
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<td>TLP35</td>
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<td>8.00</td>
<td>0.00</td>
<td>0.23</td>
</tr>
<tr>
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<td>8.00</td>
<td>0.00</td>
<td>0.24</td>
</tr>
<tr>
<td>MD2324</td>
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<td>8.00</td>
<td>0.00</td>
<td>0.23</td>
</tr>
<tr>
<td>VAX6</td>
<td>2.00</td>
<td>8.00</td>
<td>0.00</td>
<td>0.19</td>
</tr>
<tr>
<td>BAT881</td>
<td>2.00</td>
<td>8.00</td>
<td>0.00</td>
<td>0.17</td>
</tr>
<tr>
<td>SEA5</td>
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<td>8.00</td>
<td>0.00</td>
<td>0.23</td>
</tr>
<tr>
<td>RAB651</td>
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<td>8.00</td>
<td>0.00</td>
<td>0.2</td>
</tr>
<tr>
<td>BAT477</td>
<td>2.00</td>
<td>8.00</td>
<td>0.00</td>
<td>0.17</td>
</tr>
<tr>
<td>A774</td>
<td>2.00</td>
<td>8.00</td>
<td>0.00</td>
<td>0.41</td>
</tr>
<tr>
<td>G4825</td>
<td>2.00</td>
<td>8.00</td>
<td>0.00</td>
<td>0.22</td>
</tr>
<tr>
<td>G19227A</td>
<td>2.00</td>
<td>8.00</td>
<td>0.00</td>
<td>0.22</td>
</tr>
<tr>
<td>AFR475</td>
<td>2.00</td>
<td>8.00</td>
<td>0.00</td>
<td>0.2</td>
</tr>
<tr>
<td>SEQ7</td>
<td>2.00</td>
<td>8.00</td>
<td>0.00</td>
<td>0.27</td>
</tr>
<tr>
<td>AND774</td>
<td>2.00</td>
<td>8.00</td>
<td>0.00</td>
<td>0.41</td>
</tr>
<tr>
<td>MAM38</td>
<td>2.00</td>
<td>8.00</td>
<td>0.00</td>
<td>0.28</td>
</tr>
<tr>
<td>RAB665</td>
<td>2.00</td>
<td>8.00</td>
<td>0.00</td>
<td>0.26</td>
</tr>
<tr>
<td>G3513</td>
<td>2.00</td>
<td>8.00</td>
<td>0.00</td>
<td>0.23</td>
</tr>
<tr>
<td>G21212</td>
<td>2.00</td>
<td>8.00</td>
<td>0.00</td>
<td>0.23</td>
</tr>
</tbody>
</table>
Table 2.2. Summary of basal root whorl number and total basal roots in 173 *P. vulgaris* L. accessions. Genotypes were classified as 3-whorl if they had greater than an average of 2.5 whorls or as 2-whorl if they had less than 2.5 whorls. (Table from Widrig, 2004).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Root Class</th>
<th>Basal root number</th>
<th>Seed weight (g)</th>
<th>Number of genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>G19833 X DOR 364 RILs &amp; parents</td>
<td>2</td>
<td>8.09 (±0.40)</td>
<td>0.271 (±0.052)</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10.59 (±0.82)</td>
<td>0.290 (±0.064)</td>
<td>19</td>
</tr>
<tr>
<td>G19839 X G2333 RILs &amp; parents</td>
<td>2</td>
<td>8.04 (±0.25)</td>
<td>0.355 (±0.064)</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10.56 (±0.73)</td>
<td>0.398 (±0.052)</td>
<td>11</td>
</tr>
<tr>
<td>CIAT Parents &amp; Elite lines</td>
<td>2</td>
<td>8.04 (±0.32)</td>
<td>0.255 (±0.073)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10.44 (±0.71)</td>
<td>0.403 (±0.128)</td>
<td>10</td>
</tr>
<tr>
<td>Wild <em>P. vulgaris</em></td>
<td>2</td>
<td>7.34 (±0.79)</td>
<td>0.066 (±0.037)</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4.77 (±0.99)</td>
<td>0.046 (±0.017)</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 2.3. Variation in basal root angles among root whorls of shallow rooted and deep rooted genotypes. Ranges in angle were obtained from 24 plants (3 genotypes in 4 replications under low P treatments in the initial screening. Genotypes were considered shallow-rooted if the average angle of all the whorls were less than 45 degrees, deep rooted if the average angle of all whorls were more than 45 degrees from horizontal.

<table>
<thead>
<tr>
<th>Whorl position&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Average basal root angle from horizontal position, degrees</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shallow rooted genotypes</td>
</tr>
<tr>
<td>Whorl 1</td>
<td>5-20</td>
</tr>
<tr>
<td>Whorl 2</td>
<td>10-30</td>
</tr>
<tr>
<td>Whorl 3</td>
<td>20-40</td>
</tr>
<tr>
<td>Whorl 4</td>
<td>30-60</td>
</tr>
<tr>
<td>Mean of root angles</td>
<td>&lt;45</td>
</tr>
</tbody>
</table>

<sup>1</sup>Whorl position was counted from acropetal to basipetal positions.
Table 2.4. Percentage reduction of total root length by low phosphorus treatment among genotypes from the two whorl classes. Each value of total root length is the mean of 4 replicates. Plants were grown in the greenhouse harvested at 28 DAP. Values followed by same letter are not statistically different. Significant differences in percentage of total root length reduction was observed between genotypes with 2 whorls and genotypes with 3 whorls (genotype class).

<table>
<thead>
<tr>
<th>Whorl Class</th>
<th>Genotype</th>
<th>LP</th>
<th>HP</th>
<th>% Reduction</th>
<th>Class Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 whorls</td>
<td>76</td>
<td>120.52</td>
<td>296.02</td>
<td>59.3a</td>
<td>Class Mean</td>
</tr>
<tr>
<td>2 whorls</td>
<td>73</td>
<td>130.77</td>
<td>313.43</td>
<td>58.3a</td>
<td></td>
</tr>
<tr>
<td>2 whorls</td>
<td>13</td>
<td>145.53</td>
<td>391.05</td>
<td>62.8a</td>
<td>60.1a</td>
</tr>
<tr>
<td>3 whorls</td>
<td>62</td>
<td>179.59</td>
<td>308.00</td>
<td>41.7b</td>
<td></td>
</tr>
<tr>
<td>3 whorls</td>
<td>51</td>
<td>206.72</td>
<td>391.04</td>
<td>47.1ab</td>
<td></td>
</tr>
<tr>
<td>3 whorls</td>
<td>43</td>
<td>232.85</td>
<td>420.40</td>
<td>44.6 b</td>
<td>44.5b</td>
</tr>
</tbody>
</table>

Table 2.5. Shoot phosphorus content in two whorl classes of common bean genotypes grown under low P (5 ppm) and medium P (19 ppm) in the field. Values in parenthesis represent standard errors, with 4 replicates.

<table>
<thead>
<tr>
<th>P-Treatment</th>
<th>Shoot phosphorus content, mg/plant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whorl class 1: 2 whorls</td>
</tr>
<tr>
<td>Low P</td>
<td>3.84 (±0.08)*</td>
</tr>
<tr>
<td>Medium P</td>
<td>7.52 (±0.38)</td>
</tr>
</tbody>
</table>
Table 2.6. Results of linear models comparing specific treatments of the effects of various growth parameters.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Shoot dry weight, mg/plant</th>
<th>Total leaf area, cm³/plant</th>
<th>P content, mg/plant</th>
<th>Basal root whorl number</th>
<th>Root hair length, cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F-ratio</td>
<td>df</td>
<td>F-ratio</td>
<td>df</td>
</tr>
<tr>
<td>Phene</td>
<td>1</td>
<td>10.20**</td>
<td>1</td>
<td>6.78***</td>
<td>1</td>
</tr>
<tr>
<td>P level</td>
<td>1</td>
<td>49.85***</td>
<td>1</td>
<td>28.60***</td>
<td>1</td>
</tr>
<tr>
<td>Phene*</td>
<td>1</td>
<td>0.43</td>
<td>1</td>
<td>0.34</td>
<td>1</td>
</tr>
<tr>
<td>P level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.91</td>
<td>0.86</td>
<td>0.97</td>
<td>0.99</td>
<td>0.90</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Effect</th>
<th>Root length at 0-15 cm depth, cm</th>
<th>Root length at 15-30 cm depth, cm</th>
<th>Total core root length, m</th>
<th>Basal Root Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F-ratio</td>
<td>df</td>
<td>F-ratio</td>
</tr>
<tr>
<td>Phene</td>
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### Significance Levels

- *** Significant at p ≤ 0.05
- ** Significant at p ≤ 0.01
- * Significant at p ≤ 0.1

P Level-Soil phosphorus availability: Medium P (18 mg/L), and Low P (5 mg/L).
### Appendices

**Appendix 2.1.** Summary of wild *P. vulgaris* L Whorl and Basal Root Number screening results. Statistical analysis showed significant differences in seed weights and interaction between whorl and weights)

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Appendix 2.2. Summary of wild *P. vulgaris* L Whorl and Basal Root Number screening results. Average whorl and basal root number are based on the screening of 5 replicates (5 seeds) per genotype.

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Chapter 3

Quantitative Trait Loci (QTL) Analysis of Basal Root Whorl Number in Common Bean (*Phaseolus vulgaris* L.)

Miguel, M.A.¹, M. W. Blair²,³, K. M. Brown¹, N. Hurtado³, and J. P. Lynch¹,⁴

¹Department of Plant Science, The Pennsylvania State University, University Park, PA 16802, USA.
²Department of Plant Breeding, Cornell University, Ithaca, NY 14853, USA
³CIAT, Apartado Aereo 6713, Cali, Colombia.
⁴Corresponding author. Email: JPL4@psu.edu

Key words: QTL analysis, basal root whorls, phosphorus, common bean.

Abstract

Basal Root Whorl Number (BRWN) is a root architectural trait in common bean that plays an important role in soil exploration and resource acquisition. BRWN varies from one to five among bean genotypes, and is an important determinant of basal root number (BRN), with each whorl typically forming four basal roots. The objective of this study was to perform a quantitative trait loci (QTL) analysis for BRWN and BRN using two populations of recombinant inbred lines (RILs) developed from the crosses DOR364 x G19833 and G2333 x G19839. Phenotypic data on the number of basal root whorls and number of basal roots was measured on seedlings 3 days after imbibition. QTL analysis for basal root whorl number and total basal root number was performed using composite interval mapping in these two populations using four phenotypic datasets. We found a total of 23 QTL associated with BRWN and BRN in the two populations. In the DOR364 x G19833 RIL population, we found 3 QTL in the first dataset with one QTL controlling 14.6% of the variation. For the fourth dataset, we found 7 QTL with one QTL controlling 23.8% of the variation in BRWN. For BRN, we detected 3 QTL in the 2005 dataset with one QTL controlling 13.7% of the variation. In the fourth dataset, we found 7 QTL on 5 linkage groups. One of the QTL on linkage group B7 controlled 25.9% of all the variation for BRN in that population. Variability in BRWN in the G2333 x G19839 RIL population was controlled by only one locus on linkage group B3. For basal root number in the DOR364 x G19833 RIL population, we detected 4 QTL on B3, B6 and B7 in the first trial, and two QTL on B2 in the second trial. No QTL was found in the third trial.
For the fourth dataset we found one QTL in linkage group B3 controlling 19.3% of the variation in BRWN. This proportion of variation explained by relatively few loci suggests that the potential for genetic manipulation of these traits via these locus is very good. As we have observed in the results of QTL analysis of phenotypic data from four different data sets over the years, QTL were detected in different parts of the genome. It appears that there are several regions which contain QTL or genes that can contribute to the development of basal roots.

**Introduction**

Common bean is the most important food legume on earth (Lynch and Beebe 1995). Although in terms of production, groundnut (*Arachis hypogaea* L.) exceeds common bean (22 millions of metric ton against 19 million metric ton worldwide), bean is more important for food security for many people, especially in developing countries (ICRISAT, 1995). For over 500 million people in Latin America and Africa, common bean is an important source of nutrients and dietary protein (Broughton et al. 2003). Low soil fertility is a primary constraint to bean production in many developing countries, affecting at least 80% of global bean production (CIAT 1987; Wortmann et al., 1998). Of the several edaphic stresses affecting bean production, drought, low phosphorus and soil acidity are among the most important factors, affecting large areas in Africa and Latin America. In southern and Eastern Africa alone, more than 85% of all bean production area is affected by low phosphorus availability (CIAT, 1998).

Plants display a variety of adaptations to low phosphorus availability, including changes in root architecture, morphology, anatomy, increased production and secretion of root exudates, increased proliferation and elongation of root hairs, modification of carbon
metabolism and alternative respiratory pathways, and enhanced expression of Pi transporters (Lambers et al. 2006; Lynch and Brown, 2001). Root architectural traits that enhance topsoil foraging are particularly important for phosphorus acquisition since phosphorus is relatively immobile in soil and it is typically more available in the topsoil and declines substantially with depth (Lynch and Brown 2001).

Two specific root architectural traits related to basal root whorls and basal root number (BRWN and BRN, respectively) are of interest as they influence plant ability to acquire phosphorus in infertile soil. An increased number of basal root whorls is expected to be strongly associated with an increased number of total basal roots in a given genotype (Basu et al, 2007). Basal roots play an important role in nutrient uptake. When basal roots are shallow, they can spread nearly horizontally and explore more topsoil, making their importance even greater for nutrient acquisition efficiency, especially in phosphorus stressed soils characteristic of most of agroecosystems worldwide. In addition to providing an increased number of basal roots, the increased number of root whorls promotes root dispersion toward topsoil foraging and an increased soil volume being exploited by the plant, and therefore, minimizing the inter-root spatial competition within the roots of the same plant for the nutrient (Ma et al., 2001). Genotypes seem to have larger or smaller angles depending on the number of whorls. Typically genotypes with increased BRWN exhibit basal roots, arising from uppermost whorls, with large angles. Thus, the distribution of basal roots within the soil would be skewed towards shallower or deeper soil layers by larger or smaller growth angles, and the vertical distribution would be greater in genotypes with a larger range of angles (Basu et al., 2007).
Phenotypic screening of both wild and cultivated accessions of common bean suggest large genetic variation in basal root whorl number (Widrig, 2005). In addition, screening of recombinant inbred lines also showed variation in root whorl number. Basal root whorl number in initial screening varied from one to four whorls and sometimes, up to five whorls per genotype (Widrig, 2005).

Marker-assisted breeding utilizing QTL has become a useful tool in crop improvement including in common bean (Blair et al. 2010). Genetic maps, based on molecular markers, have become available over the last two decades, and represent an alternative approach to traditional phenotypic selection of target traits. Typically a QTL study includes an accurate phenotypic evaluation of an adequately large mapping population, its molecular mapping profile and a statistical analysis of the association between a phenotype and a marker genotype (Tuberosa et al. 2002). Marker assisted selection through correlation analysis between genotype and phenotypic data in QTL mapping, is an option to select for root traits that are difficult or time consuming to evaluate phenotypically.

Common bean varies in basal root whorl number, and it is possible that this trait is heritable. In addition, this trait can be related to phosphorus acquisition efficiency. Therefore, breeding programs could be aimed to select for increased numbers of basal root whorls to produce P-efficient lines of bean, using molecular tools.

The objectives of this study were to identify quantitative trait loci controlling variation in basal root whorl number and basal root number in two populations of recombinant inbred lines of common bean, and estimate the extent of phenotypic variation and heritability for these traits. The first RIL population was developed from the
cross between DOR364, with 2 whorls, and G19833 with 3 whorls. The second RIL population was developed from the cross between G2333, with 2 whorls and G19839, with 3 whorls.

**Materials and Methods**

**Plant Material**

A total of 171 recombinant inbred lines (RIL), from two populations developed at CIAT (International Center for Tropical Agriculture) were evaluated in this research. The first populations consisted of 84 F$_8$ generation RILs developed by single seed descent from the cross G2333 x G19839 and described in Ochoa et al. (2006). G2333 (‘Colorado de Teopisca’) is a climbing, small red seeded Mexican landrace of common bean, belonging to the Mesoamerican gene pool (Singh et al. 1991) with a type IV growth habit and G19839 is a large, yellow and black-mottled seeded Peruvian landrace with type III growth habit that belongs to the Andean gene pool (Singh et al. 1991). The two parental genotypes are landraces that have shown contrasting phenotypes for BRWN among germplasm accessions previously evaluated in three days after germination in the cigar roll culture system (Basu et al., 2007).

The other set of 87 RILs was also developed by CIAT and the RILs were obtained from F$_{11}$ progenies of the initial cross between DOR 364 and G19833. G19833 is a Peruvian land race (of the Andean gene pool) with a bush indeterminate 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 and Lynch 1998), formation of shallow basal roots suitable for phosphorus uptake from upper soil horizons where usually phosphorus is concentrated (Bonser et al. 1996) and high BRWN (Basu et al., 2007).

Meanwhile, DOR 364 despite its high yielding characteristics this genotype has been demonstrated to be phosphorus inefficient due to its root characteristics (Liao et al. 2004).
QTL mapping

Molecular markers used in the DOR364 x G19833 population include a total of 361 simple sequence repeat (SSR) markers (Galeano et al., 2011); 5 random amplified polymorphic DNA (RAPD) primers (Operon Technologies Inc., Alameda, CA), 146 Single Nucleotide Polymorphism (SNP) (Galeano et al., 2011), 33 Restriction Fragment Length Polymorphism (Blair et al., 2003) and 16 sequence tagged site markers (STS) (McClean et al. 2002).

The linkage map of G2333 x G19839 population include 113 simple sequence repeat (SSR) markers; 50 random amplified polymorphic DNA (RAPD) markers (Operon Technologies Inc., Alameda, CA), 16 Single Nucleotide Polymorphism (SNP) markers (Galeano et al., 2011), 3 Sequence Characterized Amplified Region (SCAR) and 11 sequence tagged site markers (STS).

RAPD markers were named according to the primer used and the molecular weight of the band as a fraction of thousand kb. PCR amplifications for the SSR and SNP markers were performed in a PTC-200 thermocycler (MJ Research Inc., Watertown, MA) in a reaction mix volume of 12 μL containing 20 ng of bean genomic DNA, 0.1 μM of each of forward and reverse primers, 125 μM of each dNTP, 1 unit of Taq polymerase, and 10X PCR MgCl2 buffer. PCR amplification, PAGE electrophoresis and silver staining detection conditions were as described in (Blair et al. 2003). SSR markers for the RILs were multiplexed with two to four loads per polyacrylamide gel.

The PCR amplifications for the RAPD, and STS markers were conducted in a PTC-100 thermocycler (MJ Research Inc., Watertown, MA). The PCR reaction mix volume was 25 μL containing 10 ng of bean genomic DNA, 0.8 μM of primer, 200 μM of each dNTP, 1 unit of Taq polymerase, and 10X PCR buffer with 10 mM of Tris-HCl (pH 7.2), 50 mM of KCl, and 2.5 mM of MgCl2. RAPD markers were named according to the primer used and the molecular weight of the band as a fraction of thousand kb.

Marker amplifications were standardized to 38 cycles at 91 °C for 15 s; 42 °C annealing for 15 s.; and 72 °C extension for 1 min. The last cycle was followed by 5 min. extension at 72 °C. A total of 5 μL of PCR products were loaded onto 1.5% (w/v) agarose gels with ethidium bromide and run in horizontal electrophoresis chamber at 240 W for
30 min. in which 150 mL of TBE 0.5x buffer was added. PCR amplification products were visualized under UV light and photographed with a Polaroid GelCam DS-34 camera (Waltham, MA, USA).

Genotypic data analysis

Linkage analysis was conducted using the software MAPMAKER/EXP 3.0 for Windows (Lander et al. 1987) set to the Kosambi mapping function. In order to create a framework map, a subset of markers with a LOD of 6.0 and a maximum distance of 20 cM were identified and then used for the placement of additional markers based on the most-likely interval using the ‘try’ command. The best marker order of the linkage groups was determined using a minimum LOD of 3.0 with the ‘compare’ and ‘ripple’ commands. Linkage groups were named according to the core reference map (Freyre et al. 1998) based on microsatellite map locations in (Blair et al. 2003).

QTL analysis was conducted with the resulting genetic map and the phenotypic means for each RIL using the computer software program WinQTL Cartographer version 2.5 for Windows (Basten et al., 2003). QTL for root traits and their combined effects were identified with Composite Interval Mapping (CIM) Parameters for CIM analysis included a forward/backward regression with a window size of 10 cM, a walk speed of 1 cM and probability thresholds of 0.05 each for the partial F-test for both marker inclusion and exclusion. The empirical thresholds for QTL detection with the CIM method were estimated using 1000 permutation tests. QTL were declared when they surpassed the threshold and were identified at the peak of the LOD profile.

Phenotypic data analysis

The evaluations were made in four different years (2005, 2009, 2010 and 2011). For the first three trials, six seeds of each RIL were germinated in a “cigar roll” culture system. Four uniform seedlings of each RIL, representing four replications were used for the first two trials, while the trial in for 2011, a total of 10 seedling replicates were used for root phenotyping and QTL analysis. Seeds were sterilized in 0.5% NaOCl for 1 minute and washed twice in deionized H₂O before germination. Then, seeds were rolled
in brown germination paper (Anchor Paper, St. Paul, MN, USA.) and placed in 47.5 cm x 35 cm x 25 cm (length x width x height), plastic containers (Sterilite, Townsend, MA, USA) filled with 50 ml of 0.5 mM CaSO$_4$. The containers were placed in a germination chamber at 28 ± 1 °C for three days in the dark. On the fourth day, the seedlings were evaluated for basal root whorl number (BRWN) and total number of basal roots (BRN).

Statistical Analysis

Genotypes were screened for BRWN during four seasons (2005, 2009, 2010 and 2011). A Completely Randomized Design was used in each experiment. Significant differences among genotypes, were determined with an ANOVA for each population and for each trait, with a p<0.05. Finally Pearson correlation analysis was calculated between the variables and between the datasets for each variable across the 2009, 2010 and 2011 trials for BRWN and BRN. All analyses were performed with SAS Software.

Results

*Phenotypic Variation for BRWN*

BRWN variation in the RILs from DOR364 x G19833 showed a normal distribution (Figure 3.1, top). More than half of the 87 genotypes had two whorls and around 25% had three whorls. A small number of genotypes had one or four whorls. The frequency distribution of BRWN from the RIL population developed from G2333 x G19839 is shown in Figure 3.1, bottom. Descriptive Statistics of BRWN among recombinant inbred lines of DOR364 x G19833 grown in 2005 show that more than 60 % of genotypes had two whorls and about 38 % showed three whorls, with very few genotypes with either one or five whorls. Data for 2011 indicate that more genotypes had three whorls and there was an increase in genotypes with four whorls compared to data from 2009 and 2010.

Frequency distribution for Basal Root Number (BRN) among RILs of DOR364 x G19833, showed a skewed distribution (Figure 3.2, top). Most of the genotypes had eight basal roots, followed by genotypes with 12, 10 and seven basal roots. A few genotypes also had less than eight or more than 12 total basal roots. The RILs of G2333 x G19839
had less variation in BRN among genotypes (Figure 3.2, bottom). Genotypes had either eight basal roots or 12 basal roots in total. About 80% of the genotypes had eight roots and the rest had four to 16 basal roots, most having 12 in total.

Significant Pearson correlations were observed for the same trait measured for all genotypes across years (Table 3.4).

Seed weight varied among RILs of DOR 364 x G19833. One of the parental genotypes, G19833, had a seed weight of 0.66 g/seed, while the other parent, DOR364, had a seed weight of 0.35 g/seed. Among RILs, seed weights varied from 0.20 to 0.70 g/seed, which is evidence of transgressive segregation. Most of the RILs, however, had seed weights ranging from 0.17 to 0.40 grams, with an average of 0.35 grams.

*Genetic mapping*

Genetic mapping of the DOR364 x G19833 cross performed prior to this study obtained a total of 561 polymorphic markers that could be assigned to the genetic map, which consisted of 11 linkage groups and a total cumulative length of 2737.62 cM. Average linkage group length was 248.87 cM with an average of 51 markers per linkage group. Linkage groups were correlated with the genetic maps of (Freyre et al. 1998; Blair et al. 2003) by the mapping of systemic and single-copy markers. The average distance between marker loci on all linkage groups was 4.5 cM, showing a uniform distribution of the markers throughout the map with only a few gaps remaining. Several clusters of microsatellite markers were observed in various linkage groups but particularly on linkage groups B4, B7, and B10.

The genetic map of G2333 x G19839 had a total of 193 mapped markers in 11 linkage groups and a total map length of 1877.64 cM with an average linkage group length of 170.69 cM. The average number of markers in each group was 17.5 and the average distance between them was 10 cM.

*Quantitative Trait Loci analysis of two populations of recombinant inbred lines*

In the DOR364 x G19833 population (Table 3.3) we identified a total of 10 QTL
for BRWN of which three QTL were detected for the 2005 data on the linkage groups B3, B6 and B7; while seven QTL were detected for the 2011 data with the following distribution: two QTL on linkage group B7 and the remaining three QTL were detected on linkage groups B3, B8 and B11 respectively. In the 2011 data, seven QTL were detected on five linkage groups.

For the same population, a total of 13 QTL were detected for BRN on seven linkage groups. Of these, three QTL were the same as QTL detected in 2005, one on linkage group B3, two on linkage group B6 and one on linkage group B7. For the 2009 data, only two QTL were detected on linkage group B2. For the 2011 data, among the seven QTL detected in five linkage groups, two QTL were detected on linkage groups B2 and B7, while the remaining three QTL were detected on linkage groups B8, B10 and B11. No QTL were detected in the 2010 data set for BRN.

Among the four QTL detected in the 2005 data for BRN from the DOR364 x G19833 population, one of the QTL explained 13.7% of the variation and the other three QTL explain 11.6%, 9.4% and 9.2% respectively. The two QTL detected in the 2009 data explained 16.7% and 13.8% of the phenotypic variation in BRN. Of the seven QTL for BRWN that were detected in the 2011 data, the percentage of variation explained by individual QTL ranged from 7.4% to 23.8%. In total, 23 QTL for both BRWN and BRN were detected in the DOR 364 x G19833 population.

In the G2333 x G19839 population we identified one QTL on linkage group B3. The detected QTL was associated with BRWN and explained 19.4% of phenotypic variation for this trait, and it was detected with the 2011 data set. No QTL were detected for BRN in the G2333 x G19839 RIL population with the 2011 data set (Table 3.4). This same QTL appeared to map to the same region as Brwn 3.2_2011 in the DOR 364 x G19833 population, based on flanking markers BMd1 and BM197 in the two populations. However, QTL were not stable over seasons. The use of several evaluations were meant to reveal which of the QTL were the most stable and amenable to marker assisted selection.
Discussion

There is a substantial genetic variation within common bean for plant and root growth under low phosphorus conditions (Lynch and Beebe 1995). Genotypes vary for root length (Checa and Blair, 2003), root hair length (Yan et al. 2004; Miguel 2004) adventitious roots (Ochoa et al. 2006), basal root growth angle (Bonser et al. 1996; Liao et al. 2004), and several other traits. Cultivated bean accessions are less sensitive to low phosphorus availability than their wild ancestors (Lynch and Beebe 1995). This suggests that the tolerance observed in cultivated common bean probably evolved after plant domestication where early landraces were moved from their original environment into phosphorus-deficient environments (Beebe et al. 1997; Lynch and Beebe 1995). One adaptive trait for low phosphorus availability could be an increased number of basal root whorls. When wild common bean ancestors were compared to their cultivated relatives, fewer whorls were found in wild ancestors compared to cultivated genotypes (Widrig, 2005).

Recombinant inbred lines from both populations tested in this study were developed from parents contrasting in low phosphorus soil adaptation, basal root whorl number (BRWN) and hence total basal roots (BRN). For each cross, the Mesoamerican parent (DOR 364 or G2333) had two whorls and eight basal roots on average, while the Andean parent (G19833 or G19839) had three whorls and 12 total basal roots on average. RILs resulting from these original parents showed significant variation for BRWN and for BRN.

The observation that some RILs had less than two or more than three whorls in these populations suggests that these genotypes carry combinations of genes responsible for basal root whorl traits other than those phenotypically observed from the two parents, which might still be segregating or might be an effect of a transgressive segregation. We also observed that more than 20% of the RILs in both populations tended to resemble the
G19833 and G19839 parents, which have three whorls and a total of 12 basal roots in average. This observation seems to support the idea that although the majority of cultivated beans have on average two to three whorls, a few genotypes have an increased number of basal roots, which may be important for their adaptation to unfavorable soil conditions. Field evaluation of a core collection of common bean for BRWN showed that the majority of screened genotypes exhibited two whorls; followed by genotypes with three whorls and a few genotypes had four and even fewer genotypes with five whorls (Jochua, C., 2012). Should the value of BRWN for soil resource acquisition be confirmed, this variation may be useful in bean genetic improvement.

Transgressive segregation in both directions was also observed for both traits evaluated (BRWN and BRN), indicating that neither parent carried all positive or negative alleles for these traits.

The results of the QTL analysis was promising since some of the same QTL controlling BRWN and BRN were found in different years. For example, for BRWN two QTL, one on linkage group B3 and the other on linkage group B7 were found both in the 2005 and 2011 datasets. For BRN, two QTL on linkage group B2 were found both in data from 2005 and 2009. This consistency in QTL found across seasons suggests a greater degree of stability in the expression of these QTL associated with the root traits being studied. In addition, in the present study, we found an apparent overlap in the location of QTL detected for BRWN in the recombinant inbred lines from the two populations (G2333 x G19839 and DOR364 x G19833). This was because many more QTL were found for the second population than for the first one. In summary, the phenotypes of BRWN and BRN traits in a range of individuals from each population might reflect substantial environmental effect, contrary to expectations based on evaluations carried on the parental lines. There were a few QTL that were not consistent across the three years. This inconsistency, might be due to the fact that environmental factors might affect the expression of genes responsible for the phenotypes.

As we have observed in the results of QTL analysis of phenotypic data from four different data sets over the years, QTL were detected in different parts of the genome. It
appears that there are several regions which contain QTL or genes that can contribute to the development of basal roots. In this sense, BRWN might not be as stable as it has been imagined at the outset. Once basal roots are initiated, however, there are still real differences. For example, G19833 continues to develop more basal roots than DOR364. Therefore, it appears that there might be some sort of feedback on numbers of basal roots formed. That is, once basal roots are initiated, there is some limit in response to some internal control. Otherwise, if all the genes for basal roots were expressed, there would be many more basal roots.

We also observed the contribution of additive alleles of the RILs from the parents in both populations. While a majority of the positive alleles for the QTL were derived from parents G19833 and G19839, the other paternal lines DOR 364 and G2333 also contributed some beneficial alleles for other root traits. As it has been shown in common bean (Liao et al. 2004; Yan et al. 2004) and in maize (Zhu et al. 2005), both parents are contributing to the phenotypic expression of the root traits being evaluated.

As we have observed in the results of QTL analysis of phenotypic data from four different data sets over the years, QTL were detected in different parts of the genome. It appears that there are several regions which contain QTL or genes that can contribute to the development of basal roots. This raises the question of why all regions are not expressed in any given evaluation. Perhaps there is some sort of internal feedback on numbers of basal roots formed. That is, once basal roots are initiated, there is some limit on the total number of basal roots in response to some internal control. Otherwise, if all the genes for basal roots were expressed, there would be many more basal roots. However, there are still real differences in numbers of basal roots. For example, G19833 continues to develop more basal roots than DOR364. Perhaps this difference is due variations in that hypothetical mechanism of internal control.

**Conclusions**

Studies on the utility of basal root whorl number for soil resource acquisition have been carried out both in the greenhouse and in the field (Chapter 2, of this thesis). IN low P soil, genotypes with increased number of basal roots have better performance, that is,
greater shoot dry weight, relative leaf area and phosphorus content, compared to
genotypes with reduced basal whorl number. We have identified a total of 23 QTL in
seven linkage groups in two RIL populations of common bean. We also found that a
useful proportion of phenotypic variation (both BRWN and BRN) is related to these QTL
altogether. Although variation in detected QTL was observed across seasons, some QTL
remained stable and reliable for further reference. These results also suggest that these
phenotypic traits are subject to environmental conditions. If the trait is subject to
environmental influence, a method should be devised to quantify the expression of the
trait under field conditions. However, the obtained information on QTL associated with
BRWN and BRN in both populations can be useful in developing common bean
genotypes with increased BRWN and hence BRN, which will confer better performance
compared to genotypes with few BRWN under low P availability. This QTL analysis for
basal root whorl number in two different RIL populations of common bean in three
different dataset showed some degree of consistency between the two RIL populations
and across seasons. Three QTL associated with BRWN were detected in the 2005 data set
in three linkage groups (B3, B6 and B7), while for the 2011 dataset seven QTL were
detected in five linkage groups (B2, B3, B7, B8 and B11). Although we found consistent
QTL responsible for each of the traits, we also found some inconsistencies of QTL
related to both BRWN and BRN across seasons. This suggests that some QTL controlling
BRWN and BRN might be affected by environmental conditions. The only QTL for BRN
found in 2011 dataset for the G19833 x G2333 RIL population explained 19.4% of the
variation in BRWN.

The significant major QTL found in the DOR364 x G19833 population might be
used along with some other QTL reported for other root traits in common bean, such as
total root hair length for tap root (Yan et al. 2004), adventitious rooting (Ochoa et al.,
2006), proportion of shallow basal root length and phosphorus uptake already detected in
linkage group B9 (Liao et al. 2004), as targets for a marker-assisted selection program to
incorporate multiple mechanisms of phosphorus acquisition efficiency in common bean,
important for yield increase in low phosphorus environments.
References


Figures

**Figure 3.1.** Frequency distribution of BRWN in the DOR 364xG19833 RIL (top) and in the G2333xG19839 (bottom) RIL populations. Seedlings were evaluated 3 days after germination. Each RIL was represented by 4 seeds for 2009 and 2010 data, and by 10 seeds for 2011 data.
Figure 3.2. Frequency distribution of BRN in the DOR 364xG19833 (top) and in the G2333xG19839 (bottom) RIL populations. Seedlings were evaluated 3 days after germination in lab. Average value for basal root whorl number was determined from the number of basal roots of four seedlings per RIL for 2009 and 2010 data and of 10 seedlings for 2011 data.
Figure 3.3. QTL Mapping for basal root whorl number and basal root number in composite interval mapping analysis of the DOR364 x G19833 RIL population. The group B02 is shown divided, because it’s very large and doesn’t fit completely in the page.
Figure 3.4. (Continued) QTL Mapping for basal root whorl number and basal root number in composite interval mapping analysis of the DOR364 x G19833 RIL population.
Figure 3.5. QTL Mapping for basal root whorl number and basal root number in composite interval mapping analysis of the DOR364 x G19833 RIL population.
Figure 3.5. (Continued) QTL Mapping for basal root whorl number and basal root number in composite interval mapping analysis of the DOR364 x G19833 RIL population.
Figure 3.6. QTL mapping for basal root whorl number and basal root number in composite interval mapping analysis of the G2333 x G19839 RIL population.
Tables

Table 3.1. Descriptive Statistics and one-way Analysis of Variance (ANOVA) of basal root whorl number (BNWN) and basal root number (BRN) among recombinant inbred lines of DOR364 x G19833 population.

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<tr>
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<th>Mean Square</th>
<th>F Value</th>
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<th>R-square</th>
<th>Coeff Var</th>
<th>Mean</th>
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Table 3.2. Descriptive Statistics and one-way Analysis of Variance (ANOVA) of basal root whorl number (BNWN) and basal root number (BRN) among recombinant inbred lines of G2333 x G19839 Population.

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Table 3.3. Quantitative trait loci (QTL) for basal root whorl number and basal root number in composite interval mapping analysis of the DOR364 x G19833 and G2333 x G19839 RIL populations. Values represent QTL significance (LOD) and determination coefficients explained by each QTL ($R^2$). Linkage group location and nearest marker to the peak LOD value are given for each QTL.

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1 LG = linkage group as defined by Freyre et al (1998)
2 LOD threshold based on 1000 permutations as recommended by Churchill and Doerge (1994)
3 R2 = proportion of variance explained by QTL at test site
Table 3.4. Pearson Correlation of three data sets (2009, 2010, 2011) of BRWN and BRN in D364 x G19833 population. The significant correlations are shown in grey.

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Table 3.5. Pearson Correlation of three data sets (2009, 2010, 2011) of BRWN and BRN in GG population. The significant correlations are shown in grey.

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\(^4\) Pearson correlation coefficient
\(^5\) Correlation significant (p < 0.05)
\(^6\) Number of observations
Table 3.6. Basal root whorl number (BRWN), and number of basal roots (BRN) among Recombinant Inbred Lines (RIL) of DOR364 x G19833 population of common bean. Data correspond to years 2005, 2009, 2010 and 2011.

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Chapter 4

Chapter 4

Phene synergism between root hairs and basal root growth angle for phosphorus acquisition in common bean

Miguel, M.A.¹, J. A. Postma¹², and J. P. Lynch¹*

¹Department of Plant Science, The Pennsylvania State University, University Park, PA 16802, USA.
²Current address: Forschungszentrum Jülich, IBG-2: Plant Sciences rm 203, 52425 Jülich, Germany

*Corresponding author. Email: JPL4@psu.edu

Key words: phosphorus, common bean, root architecture, root phenes, synergism, root hairs, root shallowness, basal root growth angle, breeding.

Abstract

Multiple root traits affect phosphorus acquisition, including root hair length and density (RHLD), and basal root growth angle (BRGA). Shallow BRGA is an important trait for phosphorus acquisition efficiency by enhancing topsoil foraging, since in most soils, phosphorus is concentrated in the topsoil. Root hairs substantially increase phosphorus acquisition by expanding the soil volume subject to phosphorus depletion through diffusion. We hypothesized that shallow BRGA and long root hairs are synergetic for phosphorus acquisition, meaning their combined effect is greater than the sum of their individual effects. The purpose of this study was to evaluate this hypothesis by quantifying the effect of root hairs and basal root growth angle alone and in combination among closely related genotypes. We established a set of field experiments with Recombinant Inbred Lines (RILs) of common bean (Phaseolus vulgaris L.) grouped in 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. Results revealed substantial synergism between the two phenes. Long root hairs increased shoot biomass under phosphorus stress by 89.3% while shallow roots increased shoot biomass by 57.7%. Genotypes with both long root hairs and shallow roots...
had the greatest biomass accumulation, 298% greater than short-haired, deep-rooted phenotypes. Shoot biomass and phosphorus content of genotypes with long root hairs on deep roots and shoot biomass of genotypes with short root hairs on shallow roots did not differ, but were greater than those of genotypes with short root hairs on deep roots. We conclude that the morphological phene of longer root hairs and the architectural phene of shallower basal root growth are synergetic for phosphorus acquisition.
Introduction

Phosphorus deficiency 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 are often less effective in tropical soils because of immobilization by the soil (Cathcart 1980). The development of crop cultivars with enhanced ability to acquire phosphorus is therefore an important strategy to increase agricultural productivity in low-input agroecosystems, and to reduce input requirements in intensive agriculture (Lambers et al. 2006; Lynch 2007).

Several root phenes enhance phosphorus acquisition, including root architectural phenes for topsoil foraging (Lynch and Brown 2001) such as shallow root growth angles (Ho et al. 2005), increased basal root whorl number (Lynch and Brown 2012) and adventitious rooting (Ochoa et al. 2006; Miller et al. 1998); phenes to enhance soil exploitation including root hair length and density (Yan and Lynch 1998; Bates et al. 1995; Bates and Lynch 2001); and Lambers et al. 2006), and phosphorus-solubilizing root exudates (Yun and Kaeppler 2001; Ryan et al. 2001; Bais et al. 2006; Farr 1928; Joner et al. 1995) phenes for mycorrhizal symbioses (Smith et al. 2004), and phenes that reduce the metabolic cost of soil exploration, such as root etiolation and root cortical aerenchyma (Postma and Lynch, 2011 a,b; Fan et al. 2003). It is probable that these phenes interact to influence the phosphorus acquisition of integrated phenotypes. For example root hair length, root hair density, 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 (atrichoblasts), display synergism for phosphorus acquisition in Arabidopsis (Ma et al. 2001). 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 2007).
Phosphorus availability is greater in the topsoil, with a steep decline with depth. Therefore, root architectural traits 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 a particular importance for topsoil foraging (Rubio et al. 2003; Ho 2004; Liao et al. 2001; Lynch and Brown 2001, Liao et al. 2004). These studies show that bean genotypes with smaller BRGA (i.e. shallower roots) have better performance under low phosphorus soils, showing greater shoot dry weight and tissue phosphorus content compared to genotypes with greater BRGA (i.e. deeper roots).

Another trait that is important for phosphorus acquisition is root hair length and density (Farr 1928; Bates et al. 1995; Miguel 2004). Since phosphorus mobility in soil is governed by diffusion rather than mass flow, phosphorus uptake by roots is limited by localized phosphorus depletion around the roots. Long root hairs extend the phosphorus depletion zone from the root epidermis, thereby increasing the rate of phosphorus uptake and the total amount of phosphorus accessible by the roots (Bates and Lynch 2001; Clarkson 1985; Peterson and Farquhar 1996). Many plant species increase the length and density of root hairs in response to phosphorus deficiency (Bates and Lynch 1996; Lambers et al. 2006). Root hair length and density increased phosphorus accumulation in *Arabidopsis* growing in low phosphorus conditions in the greenhouse (Bates and Lynch 2000; Ma 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* (Clarkson 1985). Genotypic variation for root hairs is associated with increased phosphorus acquisition in several species, including barley (Gahoonia et al., 1997) common bean (Yan et al. 2004; Miguel, 2004), and maize (Zhu and Lynch 2007). We hypothesize that the utilities of BRGA and RHL/D for phosphorus acquisition are synergetic. 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 P acquisition if they have long and dense hairs. Genotypes possessing long, dense root hairs on shallow roots should therefore have greater phosphorus acquisition than genotypes either with long root hairs on deep roots or with 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 potential synergism between the architectural phene of BRGA and the morphological phene of long root hairs for phosphorus acquisition by comparison of contrasting phenotypes of common bean growing in a weathered tropical oxisol soil.

**Materials and Methods**

*Genotypic selection*

Eighty-six recombinant inbred lines (RILs) developed from the cross between DOR364 and G19833 were phenotyped for root traits. Genotype G19833 is a Peruvian land race 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. 1992; Beebe et al. 1997). DOR364 is a high yielding line, with resistance to bean golden mosaic virus developed by CIAT (Cali, Colombia). Six seeds of each of the RILs were surface sterilized with 6% sodium hypochlorite for 5 min, rinsed thoroughly with distilled water and scarified with a blade. Then seeds were germinated at 28 °C for 2 days in rolled germination paper (25.5 x 37.5 cm, Anchor Paper Co., St Paul, MN, USA). The seedlings were then transferred to growth pouches soaked with nutrient solution in low phosphorus (in μM, 3000 KNO₃, 2000 Ca(NO₃)₂, 250 MgSO₄, 25 KCl, 12.5H₃BO₃, 1 MnSO₄, 1 ZnSO₄, 0.25 CuSO₄, 0.25 (NH₄)₆Mo₇O₂₄ and 25 Fe-Na-EDTA.

Pouches consisted of a sheet of 30 × 24 cm blue germination paper (Anchor Paper Co.) inserted into a polyethylene bag of the same size with evenly spaced (3 cm apart) holes for aeration. 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 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 basal root growth angle of less than 45
degrees were considered shallow-rooted genotypes, whereas genotypes with average basal root growth angles greater than 45 degrees were classified as deep-rooted genotypes. Genotypes were classified according to root hair traits from analysis of the same population from a previous study (Miguel, 2004). Three genotypes were selected to comprise each of 4 phenotypes: a) long, dense root hairs and shallow basal roots; b) long, dense root hairs and deep basal roots; c) short, sparse root hairs and shallow basal roots; and d) short, sparse root hairs and deep basal roots. In total, twelve genotypes were used in the field experiment.

Field Experiment

This study was conducted at Instituto de Investigacao Agraria de Moçambique (IIAM) at the Sussundenga research station in Manica Province (19° 19’ 02.00” S and 33° 14’ 25.24” E, 620 masl). The soil type at the research site is an Oxisol, Udox sub-order, a red loam with low pH (4.5 to 5.5). Three months before planting the soils was limed (CaCO₃) to bring the pH to approximately 6.2. The annual average precipitation is 1100 mm. The rainfall season starts in late October or early November and continues until early April. Temperatures during the growing season ranged from 14 to 28 degrees Celsius. The experiment was planted on February 1, 2010. Seeds were inoculated with rhizobia obtained at Bunda College Microbiology Lab, Malawi, shortly before planting. The experiment had medium phosphorus and low phosphorus plots. All other nutrients were kept optimal through chemical fertilization. Simple superphosphate was used as 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, 1954), suggesting that we had moderate phosphorus and low phosphorus treatments (Figure 4.1). Weed control was performed manually. Insecticides were applied as needed using Supermetrin. Sprinkler irrigation was used to maintain soil moisture content near field capacity.

Twelve genotypes categories were grown on soils with low (6 ppm) and medium (19 ppm) phosphorus availability (Olsen, 1954). Each treatment had 4 replications in a completely randomized block design. Experimental units were plots, three rows wide and
two meters long; 21 seeds were planted in each row, with spacing of 60 centimeters between the planting rows and 10 centimeters within the row (60 cm x 10 cm spacing).

Data Collection and Analysis

Data collected from the field study included shoot dry weight, leaf area, basal root growth angle, root hair length, root distribution with soil depth, and total phosphorus content in shoot tissue. Each plant sampling was performed by taking 3 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. In studies conducted in Mozambique, plant shoot samples were collected at 14, 21 and 28 days after planting (DAP). During sample collection, shoot samples were washed with dilute bleach. Shoots were dried at 60 °C for 5 days 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 disks per plant were collected from five fully expanded, mature and active leaves. The leaf disks were dried at 60 °C for 5 days for dry weight determination. Specific leaf weight (g cm⁻²) calculated from these samples were used to estimate total leaf area from shoot biomass for each plot.

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

For determination of root hair length, we stained root fragments by submerging them in 0.05% Trypan blue solution for 3 seconds. Images from the region of the section with representative presence of root hairs were taken at 40x magnification. A calibration microscale displayed on the screen was used to determine the image size in relation to
actual size at that magnification. Then root images were analyzed for root hair length, using ImageJ software (Wayne Rasband- Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA). 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 0-15 cm and 15-30 cm depths, discarding the remaining length of 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, Québec, Canada).

Growth analysis was calculated from logarithmic values of shoot dry weight data taken at 14, 21 and 28 days after planting, among the four phenotypes, with replicates each, in both low phosphorus and medium phosphorus treatments, according to Hunt et al., 2002.

*Simulation of root distribution with in 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, 2011b), a functional-structural plant model, to simulate shallow and deep bean root architecture of plants grown in soils with medium P availability (~50% growth reduction). At 28 days 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 4 times, to average out stochastic effects in the model. In total we ran 8 simulations. We used for our simulation of beans the same parameter set as was published in Postma and Lynch, 2011b. To vary the architecture we adjusted the branching angle and gravitropism of the basal roots of both basal root whorls (Table 4.4.). The phosphorus concentration in the soil solution was 6 μM. 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 (Table 4.3).
Results

Analysis of the soil samples taken in the experimental plots after the harvest showed stratified phosphorus both in low and medium phosphorus plots, with substantially more phosphorus in surface soil strata than in deeper strata (Figure 4.1).

Under low phosphorus the long-shallow phenotype had the best performance, in terms of shoot dry weight, phosphorus accumulation, and total leaf area (Figures 4.2 - 4.4). The long-deep and short-shallow phenotypes had intermediate performance under low P. These two categories did not differ significantly for shoot dry weight, phosphorus content per plant and total leaf area per plant. Short-deep phenotypes had less shoot biomass, tissue phosphorus content/plant, and total leaf area than the other phenotypes. Shoot biomass accumulation in the long-shallow phenotypes was greater than the sum of biomass increase due to each trait in isolation (Table 4.2).

Under medium phosphorus availability (i.e. treatments with applied phosphorus fertilizer but still experiencing growth reduction due to low phosphorus availability), the long-shallow phenotype had greater shoot biomass and tissue phosphorus content than the other phenotypes (Figures 4.2, 4.3, Table 4.2) The long-deep and short-shallow phenotypes did not differ when grown under medium phosphorus availability (Figures 4.2, 4.3, Table 4.2). Genotypes with shallow basal root growth angles (BRGA) had greater biomass and greater tissue phosphorus content compared to genotypes with greater BRGA (Figure 4.2 and Figure 4.3). ‘Long-shallow’ phenotypes had significantly greater leaf area compared to other phenotypes both under low and medium phosphorus availability (Figure 4.4). Synergism was also observed in relation to shoot biomass. Large RHLD 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 resulted from the two root traits appearing separately (Table 4.2).

Under low phosphorus availability, long root hairs increased phosphorus acquisition by 2.78 mg/plant compared to the baseline (Table 4.3). Shallow basal roots increased phosphorus acquisition by 2.42 mg/plant compared to the baseline (Table 4.3). Thus the additive effect on phosphorus acquisition of both phenes would be an additional
5.2 mg/plant. Genotypes possessing both long hairs and shallow basal roots actually had additional phosphorus accumulation of 8.13 mg/plant, which is 2.93 mg/plant more phosphorus than expected from the additive effects, which is evidence of substantial positive synergism (Table 4.3). Although smaller than in low phosphorus treatments, the synergetic effect was also observed in plants growing under medium phosphorus availability (298.9% vs 87.7%).

Genotypes used in this study were selected according to root hair length and basal root growth angles measured in previous phenotypic profiling. Data from plants taken at 14 days after planting confirmed the root phenotypes of the genotypes assigned to each phenotypic category (Figures 4.5 and 4.6). Long-shallow and short-shallow phenotypes had basal root growth angles ranging from <10 to 40 degrees from horizontal. Long-deep and short-deep phenotypes had greater basal root growth angles varying from 60 to 80 degrees from horizontal (Figure 4.5). Overall, these genotypes had steeper angles under medium phosphorus availability compared to low phosphorus availability. Under low phosphorus availability, long-shallow and long-deep genotypes had greater root hair length compared to short-shallow and short-deep genotypes (Figure 4.6). In addition, genotypes were evaluated for total number of adventitious roots both under low phosphorus and medium phosphorus availability. Genotypes with deep roots had slightly more adventitious rooting than shallow-rooted genotypes, but possible effects of variation in adventitious roots on phosphorus acquisition did not obscure effects of BRGA and RHLD on plant performance (Figure 4.7).

Calculated growth rates from 14 to 21 DAP showed differences among the four phenotypes both in low and medium phosphorus treatments. Under low phosphorus availability, long-shallow genotypes had statistically greater growth rate compared to the rest of categories. In addition, short-deep genotypes had lower growth rates than the three other categories. The growth rates of long-deep genotypes was not statistically different from the growth rates of short-shallow genotypes. Under medium phosphorus, growth rates of three phenotypes (Long-deep; short-shallow and short-deep) were not statistically different. However, Long-shallow genotypes had statistically greater growth rates compared to the rest of categories (Table 4.6).
Root cores were taken at 28 DAP at two soil depths, 0-15 cm and at 15-30 cm, along planting rows. Genotypes had greater root length in cores taken in medium phosphorus treatments compared to low phosphorus treatments (Figure 4.8A and B). Root length in root cores taken at 0-15 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 (Figure 4.8A). At 15-30 cm, the long-deep and short-deep phenotypes had greater root length than long-shallow and short-shallow phenotypes (Figure 4.8B). Under low phosphorus, the long-shallow phenotype had significantly greater root length than other phenotypes (Table 4.1). Since root length in soil cores taken from 0-15 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 further away from the stem. To test this hypothesis, we simulated the root distribution in virtual cores centered either 5 cm or 15 cm from the stems of shallow- and deep-rooting bean plants at 28 days after germination (Figure 4.10). Simulation results showed statistically significant differences between shallow-rooted and deep-rooted genotypes at 0-15 cm depth with samples taken 15 cm away from planting row (Figure 4.11 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 40-50 cm (Figure 4.11 bottom).

Discussion

Our results confirm that both BRGA and RHLD are beneficial for phosphorus acquisition in isolation, in agreement with previous studies (Miguel 2004; Farr 1928; Gahoonia and Nielsen 1997; Bates and Lynch 2001; Lynch and Brown 2008; Gahoonia et al. 1997; Zhu and Lynch 2006. Our results support the hypothesis that BRGA and RHLD are synergetic for phosphorus accumulation. Under low P, larger RHLD increased shoot growth 89.3% while shallow BRGA increased shoot growth by 57.7%, compared to the ‘short-deep’ phenotype. The combination of large RHLD and shallow BRGA improved
shoot growth by 298%, double that expected from an additive effect, and evidence of substantial synergism. Under low P, larger RHLD increased phosphorus content 90% while shallow BRGA increased phosphorus content by 79%, compared to the ‘short-deep’ phenotype. The combination of large RHLD and shallow BRGA improved phosphorus content by 169%, double that expected from an additive effect, further evidence of substantial synergism (Table 4.3).

We found differences in growth rates among phenotypes in low phosphorus treatments. Long-shallow phenotypes had significantly greater growth rates compared to the rest of treatments, according to the logarithmic values of shoot dry weights taken at 7, 21 and 28 DAP, seven-day time intervals calculated according to Hunt et al., 2002.

In summary, phenotypes with greater ability to acquire phosphorus had faster biomass accumulation (Table 4.5).

Phenotypic categories for RHLD and BRGA determined in seedling assays did not change with phosphorus availability or with soil conditions in the field. This result indicates that early seedling phenotyping screens would be effective in selection programs for these phenes.

The results of the SimRoot simulation of root coring between planting rows (instead of within the planting row) supports the idea that genotypes with shallower BRGA allocated much of their basal roots to the top 15 cm of the soil profile, while genotypes with greater BRGA allocated most of their roots deeper in the soil, although this was not evident in our soil coring because of sampling very close to the plants, that is, only 5 cm away from the plant, within the planting row (Figure 4. 11 bottom).

Relatively few studies have addressed interactions among phenes associated with nutrient acquisition in plants. Functional-structural modeling showed substantial synergism among four root hair phenes (length, density, initiation, and geometry) for phosphorus acquisition in Arabidopsis (Ma et al. 2001). A study on the effect of mycorrhizas in long root hair genotypes showed that both long root hairs and mycorrhizal symbiosis contributed to overall phosphorus accumulation in low phosphorus environments (Miguel, 2004). Based on the results of the present study we hypothesize the existence of synergism between mycorrhizae and basal root growth angle, since mycorrhizas improve physical access to phosphorus, and by analogy with root hairs, this
benefit would be greater in shallow rooted genotypes than in deeper-rooted genotypes. A modeling study indicated that root cortical aerenchyma may be synergetic with lateral branching frequency for P acquisition in maize (Postma and Lynch 2011a). To our knowledge, the present study is the first to empirically demonstrate synergism between architectural and anatomical phenes for soil resource acquisition.

Phosphorus acquisition is influenced by a number of root traits. A given genotype can be exhibiting one or more root traits that are associated with P acquisition. These traits can interact among themselves creating a synergetic or antagonistic effects. The performance of a phenotype in low P soils can be affected by other factors including the limitation of other resources such as water and micronutrients. For example, water availability in most tropical ecosystems is a particular concern in this context since unlike phosphorus, water availability often increases with soil depth, and this can create a tradeoff between water and phosphorus acquisition (Ho et al. 2005). Water availability also varies within and among growing seasons. Competition with neighboring plants for soil resources may affect the contribution and utility of a given root trait among phenotypes (Nord et al., 2011, Rubio et al. 2001). Because of the complexity of the interactions of various factors associated with plant performance under a given stressful condition, simulation modeling may be valuable in predicting and assessing various scenarios in order to identify key trait combinations that should be researched empirically (Postma and Lynch. 2011).

Phene synergism is important to consider in breeding strategies for improving phosphorus acquisition by crops. Genotypes with long root hairs can be crossed with genotypes with shallow roots to develop genotypes with both long root hairs and shallow roots. Such ‘trait stacking’ can be employed to develop genotypes with greater phosphorus acquisition by employing multiple mechanisms, in this case with substantial synergism. The challenge to this approach is to determine synergisms among multiple phenes in multiple potential integrated phenotypes and environments. Given the number of possible combinations of phenotypes and environments, simulation modeling will be useful in identifying the most promising scenarios.
References


Figures

Figure 4.1. Shoot dry weight among 4 phenotypes grown under low and medium phosphorus availability in the field at Sussundenga research station, Mozambique. Long-shallow genotypes had significantly greater shoot dry weight compared to the rest of root categories. Each mean is from four replicates. Under low phosphorus availability Long-shallow phenotypes had significantly greater shoot dry weight compared to the rest of categories. Error bars represent standard error of the deviation from the mean. Means followed by the same letter are not statistically significant.
Figure 4.2. Phosphorus content among 4 phenotypes grown under low and high phosphorus availability in the field at Sussundenga research station, Mozambique. Genotypes with long root hairs and shallow roots showed significantly more phosphorus content in plant tissue compared to long-shallow and short-shallow genotypes, which had more phosphorus content compared to short-deep genotypes, when grown under low phosphorus availability. Each mean is from four replicates. Error bars represent standard error of the deviation from the mean. Means followed by the same letter are not statistically significant.
Figure 4.3. Total leaf area among 12 genotypes grown under low phosphorus and high phosphorus availability in the field. Genotypes with long root hairs and shallow roots showed greater leaf area, followed by long-deep and short–shallow genotypes, which did not show statistically significant differences among them but with greater leaf areas compared to short-deep genotypes. Each mean is from four replicates. Error bars represent standard error of the deviation from the mean. Means followed by the same letter are not statistically significant.
Figure 4.4. Basal root growth angle (BRGA) among 12 genotypes grouped according to their root categories, grown under low phosphorus and medium phosphorus availability in the field. Long-deep and short deep genotypes showed greater basal root angles compared to long-shallow and short-shallow genotypes (See ANOVA table for statistics). Each mean is from four replicates. Error bars represent standard error of the deviation from the mean. Means followed by the same letter are not statistically different.
Figure 4.5. Root hair length among 4 root categories (phenotypes) of common bean genotypes grown under low phosphorus and medium phosphorus availability in the field. Long-shallow and long-deep genotypes showed greater root hair length compared to short-shallow and short-deep genotypes. Each mean is from four replicates. Error bars represent standard error of the deviation from the mean. Means followed by the same letter are not statistically different.
Figure 4.6. Total number of adventitious roots among 12 genotypes, grown under low phosphorus and medium phosphorus availability in the field. Each mean is from four replicates. Error bars represent standard error of the deviation from the mean.
Figure 4.7. Root length in 0-15 cm soil cores, among 12 genotypes grown under low phosphorus and medium phosphorus availability in the field at Sussundenga Research station, Mozambique. Under low phosphorus availability, genotypes with short root hairs and deep basal roots had less root length compared to the rest phenotypes. Each mean is from four replicates. Error bars represent standard error of the deviation from the mean. Means followed by the same letter are not statistically different.
**Figure 4.8.** Root length in 15-30 cm soil cores with 10 cm in diameter, among 4 phenotypes grown under low phosphorus and medium phosphorus availability in the field at Sussundenga research station in Mozambique. Under low phosphorus, deep phenotypes had greater root length compared to long-shallow and shallow phenotypes. Each mean is from four replicates. Error bars represent standard error of the deviation from the mean. Means followed by the same letter are not statistically different.
Figure 4.9. Correlation between basal root growth angle (BRGA) and phosphorus content in plant tissue among 12 genotypes contrasting for BRGA. A significant negative correlation between BRGA and phosphorus content in plant tissue was detected among phenotypes grown under low phosphorus availability.
**Figure 4.10.** Correlation between basal root growth angle (BRGA) and shoot dry weight among 12 genotypes contrasting for BRGA. A negative correlation was found between BRGA and shoot dry weight among genotypes, when grown under low phosphorus availability.
Figure 4.11. Graphic representation of root coring between planting rows in shallow rooted genotypes (top), and in deep-rooted genotypes (bottom), at 28 DAP, under low phosphorus availability. Coring cylinder has 10 cm diameter.
Figure 4. 12. Simulated root density of root cores taken at 0-15 cm depth and at 15 cm away from planting row (top), and root cores taken at 0-15 cm depth and at 5 cm within the planting row (bottom) of common bean genotypes. Genotypes were contrasting for basal root growth angle; root core samples were taken at 28 DAP, under low phosphorus (5 ppm) availability. Each mean is from four simulated replicates.
**Figure 4.13.** 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. Genotypes are contrasting for basal root growth angle, root core samples taken at 28 DAP, under low phosphorus (5.0 ppm) availability. Each mean is from four replicates.
Figure 4. Results of soil samples analysis taken in medium P and low P plots in the field after harvesting the experiments in red Oxisols at Sussundenga research station, Manica, Mozambique. Each mean is from 8 replicates. Means followed by the same letter are not statistically different.
### Tables

**Table 4.1.** Results of linear models comparing specific treatments of the effects of various growth parameters.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Shoot dry weight, mg/plant</th>
<th>P content, mg/plant</th>
<th>Total leaf area, cm³/plant</th>
<th>Basal root growth length, cm</th>
<th>Root hair length, cm</th>
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<tr>
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<td>df</td>
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<td>df</td>
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<td>0.497</td>
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<th>Effect</th>
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<th>Root length at 0-15 depth, cm</th>
<th>Root length at 15-30 cm depth, cm</th>
<th>Total Core Root length, cm</th>
<th>Correlation BRGA *P content</th>
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*** Significant at p ≤ 0.05
** Significant at p ≤ 0.01
P Level-Soil phosphorus availability: Medium P (18 mg/L), and Low P (5 mg/L).
Table 4.2. Percentage increase of shoot dry weight (SDW) 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 P in the field. Plant samples were collected at 28 DAP. Each value in shoot dry weight of genotype is a mean value of 4 replicates. Percentages were calculated from the average shoot dry weight of phenotype D (8.88 g=100% for low P, and 22.66 g=100% for medium P). The following formula was used to calculate the percentage of SDW increase: \( \% X = \frac{\text{Avg. SDW of X} \times 100}{\text{Avg. SDW D}} - 100\% \), where X= phenotype. See ANOVA below for significance.

<table>
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<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>Low P SDW (g)</th>
<th>Low P Avg. SDW (g)</th>
<th>% Increase</th>
<th>Medium P SDW (g)</th>
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<td></td>
</tr>
<tr>
<td></td>
<td>DG27</td>
<td>7.74</td>
<td>8.88c</td>
<td>0</td>
<td>22.86</td>
<td>22.66b</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 4.3. Phene synergism for phosphorus accumulation among genotypes with contrasting root hair length/density, and basal root growth angle, grown under low and high phosphorus availability. Synergism was calculated as the gain in P content as the result of the presence of both traits that led to the additive effect on phosphorus accumulation. Genotypes with only one advantageous root trait (long root hairs) had an additional accumulation of phosphorus equal to 5.89 mg/plant. Genotypes with the other advantageous root trait (short-shallow) had additional accumulation of phosphorus equal to 2.85 mg/plant. When both advantageous root traits are present (long root hairs and shallow roots), the additional accumulation of phosphorus is equal to 22.43 mg/plant, which shows an extra gain of phosphorus content of 13.65 mg/plant, due to the additive/synergistic effect of these two root traits.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Phosphorus Treatment</th>
<th>Adj. P content due to the phene (mg)</th>
<th>Synergetic effect (difference between additional P minus the sum of additional P acquired due to each phene)</th>
<th>Adj. P content due to the phene (mg)</th>
<th>Synergetic effect (difference between additional P minus the sum of additional P acquired due to each phene)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low P</td>
<td></td>
<td></td>
<td>Medium P</td>
<td></td>
</tr>
<tr>
<td>Long-shallow</td>
<td>11.19 a</td>
<td>8.13</td>
<td>2.93</td>
<td>29.51 a</td>
<td>22.43</td>
</tr>
<tr>
<td>Long-deep</td>
<td>5.84 b</td>
<td>2.78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short-shallow</td>
<td>5.84 b</td>
<td>2.42</td>
<td></td>
<td>9.93 b</td>
<td>2.85</td>
</tr>
<tr>
<td>Short-deep</td>
<td>3.06 c</td>
<td>0</td>
<td></td>
<td>7.08 b</td>
<td>0</td>
</tr>
</tbody>
</table>

Within phosphorus treatment column, values followed by the same letter are not significantly different.
Table 4.4. Parameters used for simulating deep and shallow bean root architecture. Branching angles are in degrees from horizontal, gravitropism is a unitless factor which is 0 for no gravitropism and more negative for stronger gravitropism. For the gravitropism a univariate distribution was used which was sampled for each root segment. Minimum and maximum values are given.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Shallow RSA</th>
<th>Deep RSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branching angle of basals roots of lower whorl</td>
<td>10</td>
<td>45</td>
</tr>
<tr>
<td>Branching angle of basals roots of upper whorl</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td>Gravitropism of basal roots of lower whorl</td>
<td>-0.02 to -0.01</td>
<td>-0.08 to -0.04</td>
</tr>
<tr>
<td>Gravitropism of basal roots of upper whorl</td>
<td>-0.01 to -0.005</td>
<td>-0.04 to -0.02</td>
</tr>
</tbody>
</table>
Table 4.5. Growth analysis of four phenotypes grown under low and medium phosphorus availability. GR= growth rates at a given period, expressed in grams of SDW/week. Values followed by the same letter in the each of column section a not statistically different. Each value is mean from four replicates. Significance is at 5% probability.

<table>
<thead>
<tr>
<th>Phene</th>
<th>Mean log of Shoot dry weight (g), Low P</th>
<th>Mean Growth rates (Log dW/dt, g/week), Low P</th>
<th>Low P</th>
<th>Low P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14 DAP</td>
<td>21 DAP</td>
<td>28 DAP</td>
<td>G_R1</td>
</tr>
<tr>
<td>Long-shallow</td>
<td>1.04</td>
<td>1.30</td>
<td>1.72</td>
<td>0.037 a</td>
</tr>
<tr>
<td>Long-deep</td>
<td>0.90</td>
<td>1.04</td>
<td>1.30</td>
<td>0.051 b</td>
</tr>
<tr>
<td>Short-shallow</td>
<td>0.84</td>
<td>1.15</td>
<td>1.45</td>
<td>0.044 ab</td>
</tr>
<tr>
<td>Short-deep</td>
<td>0.70</td>
<td>1.26</td>
<td>1.48</td>
<td>0.049 ab</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phene</th>
<th>Mean log of Shoot dry weight (g), Medium P</th>
<th>Mean Growth rates (Log dW/dt, g/week), Medium P</th>
<th>Medium P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14 DAP</td>
<td>21 DAP</td>
<td>28 DAP</td>
</tr>
<tr>
<td>Long-shallow</td>
<td>1.19</td>
<td>1.56</td>
<td>1.88</td>
</tr>
<tr>
<td>Long-deep</td>
<td>1.11</td>
<td>1.36</td>
<td>1.76</td>
</tr>
<tr>
<td>Short-shallow</td>
<td>1.05</td>
<td>1.32</td>
<td>1.72</td>
</tr>
<tr>
<td>Short-deep</td>
<td>1.00</td>
<td>1.22</td>
<td>1.58</td>
</tr>
</tbody>
</table>
VITA
MAGALHÃES AMADE MIGUEL

Date of birth: January 1st, 1970; Place of birth: Nampula, Mozambique.
Marital status: Married to Benência José Nhacume Miguel.
Current Position and Address:
Researcher, Agricultural Research Institute of Mozambique (IIAM). Sussundenga Research station. a/c DPA-Manica, P. O. Box. 42, Pigivide Avenue, 678, Chimoio, Manica, Mozambique.
E-mail: <mam1041@psu.edu>, <magalhaes_amade@hotmail.com>

Education:
Ph. D. Candidate, Pennsylvania State University (Expected: August, 2012).
Advisors: Jonathan P. LYNCH, Kathleen BROWN, Roger KOIDE and Barbara CHRIST.

M. Sc., Pennsylvania State University (2004), USA.
Advisors: Jonathan P. LYNCH, Kathleen BROWN and Roger KOIDE.

B. Sc., Agronomy/breeding, Kazakh State Agrarian University (1996), Alma-Ata, Kazakhstan. Advisor: Nurgasin NURGASINOV, Professor of Plant Breeding.

- Researcher at Agricultural Research Institute of Mozambique (IIAM), 1997- present.
- Visiting scholar, Brazilian Agricultural Research Company (EMBRAPA), 1997-1998;
- Visiting researcher (MSc. thesis research), University of Costa Rica, San Jose, Costa Rica, 2003.
- Visiting scholar at CIAT-Colombia, August 2006.
- co-PI of four PSU/IIAM joint research projects funded by the McKnight Foundation, IAEA, GCP, and DGP CRSP/USAID (2006-present).

Publications: