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DEPLOYING ROOT TRAITS FOR AFRICAN BEAN BREEDING

A Dissertation in

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ABSTRACT

Common bean is the most important grain legume for direct human consumption in the world. It provides dietary protein and minerals for millions of people in developing countries. Low Phosphorus (P) availability and drought are major constraints to bean production in many productions region in Africa and Latin America. Genotypic adaptation to drought and low soil P availability is associated with phenotypic variation in root architecture. Plants evolved a wide range of adaptations to enhance P and water acquisition from the soil. In this dissertation, we describe the importance of root traits for resource acquisition and the used of root phenotyping and selection as an alternative for improving crop adaption to drought and low P stresses. Chapter 1 presents the general introduction of common bean as a legume crop with emphasis on the importance of root architecture for water and nutrient uptake from the soil, and the genetic basis of some root traits. In Chapter 2 we developed a rapid, simple and inexpensive method for field phenotyping of root phenes of common bean. With this method twelve architectural root phenes from one root crown can be evaluated in two minutes. The field phenotyping method we developed, *Shovelomics*, should have utility for bean breeding for low P and drought tolerance in developing countries of Africa and Latin America. In chapter 3, we describe a large and diverse genetic variation in root phenes of common bean from Andean and Mesoamerican gene pools. Large variation among genotypes within gene pools, genotypes within race, and genotypes within country of origin were detected. Genotypes with root traits associated with adaptation to low P availability were found in both gene pools, while traits associated with adaptation to drought stress were mostly found in the Mesoamerican gene pool. Our findings indicate that useful root traits for breeding for edaphic stresses were identified in both Andean and Mesoamerican gene pools. In Chapter 4, we worked with root hair traits, and longer and denser root hairs are associated with P efficiency in crops. We found large genetic variation in root hair length from basal roots within bean populations derived from parents contrasting in root hair traits. In addition, we estimated the heritability of root hair length from basal roots using parent-offspring regression analysis, and we found moderately high heritability of root hair length in two populations. This result has implications for strategies used for selection for longer and denser root hairs. The relatively high heritability suggests that considerable progress may be expected from selection for longer root hairs in segregating bean populations. Breeding for longer and denser root hairs could enhance acquisition of P in low P soils in Africa and Latin America. Breeding for multiple root phenes could enhance acquisition of multiple soil resources.

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DEDICATION

To Soares Neron, my little son,
To Neill and Nelma, for your inspiration as women,
To Soares, my husband.

Chapter 1

Introduction

The Common bean

Common bean (*Phaseolus vulgaris* L.) is one of the priority legume crop in Africa and Latin America and the Caribbean (FAOSTAT, 2011; CGIAR, 2012), and it is the main source of dietary protein and minerals for millions of people in both developing and developed countries (CIAT, 2001). Beans are cultivated in a diverse range of climates, and the world production is estimated to exceed 23 million metric tons (Broughton et al., 2003). The major bean production regions include Brazil, the Andes region, Central America, the Caribbean, North America and Africa (Schoonhoven and Voysest, 1989; Pachico, 1989). In developing countries, beans are mostly produced on small landholdings in association with other crops for family consumption, while in developed countries beans are cultivated on a large scale for commercial market. In Africa, production of beans is concentrated in the cool highlands of central and tropical eastern Africa and lowlands of northern and southern Africa where beans are grown during the cool season with irrigation (Allen et al., 1989). Among tropical regions in the world, the major bean production and consumption occur in Latin America (Schoonhoven and Voysest, 1989). Morphological and biochemical traits divide beans into two geographically distinct gene pools, Andean and Mesoamerican, which correspond to the centers of bean domestication (Gepts, 1988; Pachico, 1989; Gepts and Debouck, 1991; Singh et al., 1991a; Singh et al., 1991b; Singh et al., 1991c; Noradi et al., 1992; Broughton et al., 2003; Zizumbo-Villarreal et al., 2005). The two gene pools reflect multiple events of domestication within distinct wild populations (Gepts and Bliss, 1986, Beebe et al., 2001). Mesoamerican genotypes predominate in Mexico, Central America, and Brazil, all accounting for approximately 84% of the production in Latin America (Beebe et al., 2000). Andean genotypes are found in Andean countries of Colombia, Ecuador, Peru, Bolivia and Argentina (Tohme et al., 1996; Beebe et al., 2001). Andean genotypes are also cultivated in Brazil, Mexico and the Caribbean, temperate climates of North America and Europe (Beebe et al., 2001). In Africa, production of beans is concentrated in the cool highlands of central and tropical eastern Africa and lowlands of northern and southern Africa, where beans are grown during the cool season with irrigation (Allen et al., 1989). Mesoamerican beans such as small whites are

important in regions of Ethiopia and South Africa while Andean types are found in Central and southern Africa (Wortmann et al, 1998).

Cultivated beans are subdivided into races based on morphological and ecological adaptation. Mesoamerican beans are classified into four races: Mesoamerican, Durango, Jalisco and Guatemala (Singh et al., 1991a and Beebe et al., 2000 and 2001), and Andean beans are subdivided into three races: Nueva Granada, Peru and Chile (Singh et al., 1991a, Beebe et al., 2001). The average yield of bean is about 800 Kg/ha in Latin America, and 600 Kg/ha in developing African countries (Lynch, 2007), these values are below the average bean yield potential that is estimated as 5800 Kg/ha (Lynch, 2007).

Low soil fertility and drought are primary constraints to bean production in many developing countries, affecting more than 80% of the global production (Wortmann and Allen, 1994; Lynch, 1997; Wortmann et al., 1998; Raghothama, 1999; CIAT, 2001; Lynch, 2007). More than 50% of the bean production zones in Africa and Latin America have serious soil fertility problems (Lynch, 2007). Plant adaptation and productivity in a particular environment is primary determined by the ability of the species to obtain resources. Root architecture is an important factor in determining acquisition of soil resources (Lynch, 1997; Lynch and Brown, 2001; Lynch, 2007). For instance, drought tolerance in beans has been associated with deeper roots, while acquisition of immobile nutrients such as phosphorus (P) has been associated with better topsoil foraging (Lynch and Brown, 2001; Ho et al., 2005; Lynch, 2005). Consequently, genetic variation in root architecture among and between species is related to adaptation and productivity in specific environments (Lynch, 2005). Thus, root architectures that result in root proliferation in the topsoil will increase P acquisition, while architectures with deeper and extensive roots will explore deeper soil horizons where water and mobile nutrient are available.

Root traits and phosphorus acquisition

Phosphorus availability is of particular concern in weathered and volcanic soils of the humid tropics and subtropics and in many sandy soils of the semiarid tropics, where yield is affected by lack of available inorganic P. Phosphorus can form complexes with iron and aluminum oxides that make P unavailable to plants (Vance et al., 2003). One alternative to limited P availability is the application of fertilizers. The use of P fertilizers is often not efficient

since P can be immobilized in the soil and became unavailable. Excessive fertilizers that are not used by plants can be removed by erosion, runoff and leaching. High concentrations of P in aquatic systems result in eutrophication and degradation of the environment (Raghothama, 1999). In addition, most farmers in developing countries cannot afford fertilizers (Quiñones et al., 1997, Borlaug, 2006) although they know the benefit of fertilizers.

Plants have evolved a wide range of adaptations to enhance P acquisition from the soil (Lynch, 1995; Lynch and Brown, 2001). Adaptability to low P environments that enhance topsoil exploration include shallow basal root growth angle (Bonser et al., 1996; Lynch and Brown 2001; Rubio et al., 2003; Ho et al., 2004; Zhu et al., 2005b), adventitious rooting (Miller et al., 2003; Ochoa et al., 2006), lateral rooting (Zhu et al., 2005a) and elongation (Borch et al., 1999), root hair length and density (Bates and Lynch, 1996; 2000; and 2001; Gahoonia et al., 1997; Miguel, 2004), aerenchyma formation (Fan et al., 2003) and reduced root respiration (Nielsen et al., 2001). Field phenotyping for identification of genotypes with root traits adapted to low P availability would be important for breeding programs for development of P efficient crops.

Genetic variation of root traits

The use of genetic diversity in root traits for crop improvement is a suitable approach to improve yields, and seed from improved varieties has larger possibilities of reaching farmers in rural areas in developing countries than fertilizers. Interest in root architecture as a criterion for selection for crop adaptation to edaphic stresses has increased (Lynch and Brown, 2001; Vance et al., 2003; Lynch, 2007; Manschadi et al., 2008; Ramaekers et al., 2010; Coudert et al., 2010). Genetic variability in root traits among genotypes in different crops have been reported (Bonser et al., 1996; Gahoonia et al., 1997 and 2005; Miller et al., 2003; Rubio et al., 2003; Zhu and Lynch 2004; Zhu et al., 2005a and 2006; Ochoa et al., 2006; Burton, 2010; Bayuelo-Jiménez et al., 2010). Widrig (2005) reported variation in basal root whorl number (BRWN) and basal root number in bean. Miguel (2004) and Vieira et al. (2007) reported variation in root hair length and density in bean. Ochoa et al. (2006) reported variation in adventitious root number in the field. Sarker et al. (2005) reported variation in taproot length and number of lateral roots in lentil. Trachsel et al. (2010) reported variation in root traits in maize crown and Burton (2010) in anatomical and architectural root traits of maize.

Roots are crucial to plant performance and yield; however, roots receive little attention in plant breeding. Root traits can be used to improve tolerance to edaphic stresses such as nutrient deficiency, drought and salinity (Fageria et al., 2008). Incorporation of root phenes into plant breeding programs would be useful for crop improvement. The role of root traits in P uptake has been reported (Gahoonia et al., 1997; Ma et al., 2001; Liao et al., 2001; Zhu and Lynch, 2004; Ochoa et al., 2006, Lynch, 2005). Root hairs are subcellular extensions of root epidermal cells. Root hair proliferation and elongation increase the volume of soil exploited by plants with low carbon cost. Several studies have reported that in crop species, genotypes with long root hairs acquire more P (Gahoonia et al., 1997; Gahoonia and Nielsen, 1997; Yan and Lynch, 1998; Ma et al., 2001; Wang et al., 2004; Bates and Lynch 1996 and 2000). Bates and Lynch (2000) reported that root hairs are a cost-efficient adaptation to low P when compared mutant and wild type Arabidopsis. Genotypes with long and dense root hairs could be developed and deployed in regions with low P availability.

In addition, mechanisms of inheritance of root traits have been reported. Wang et al. (2004) studied the heritability of root hair traits in RILs of soybean and found low heritability for root hair density from basal roots (27.32%), tap roots (31.04%) and total roots (33.97%); and high heritability for root hair length from basal roots (57.85%), tap roots (59.18%) and total roots (60.98%). In beans, Araújo et al. (2005) found high to moderate broad-sense heritability for root area, root length and root mass, and P content. Narrow-sense heritability ranging from low to high was detected for adventitious root traits (Ochoa et al., 2006). The knowledge that most root phenes are genetically controlled emphasizes the need of incorporating root phenes into plant breeding programs for crop improvement.

Quantitative Trait Loci (QTL) controlling root traits in crops have been reported. QTL can be used in marker-assisted selection for screening traits of genotypes (Collins et al., 2008). In roots, QTL have been identified in maize for root hair length associated with low and high P (Zhu et al., 2005c), lateral rooting (Zhu et al., 2005a), seminal root length associated with low and high P (Zhu et al., 2006). Trachsel et al. (2009) identified QTL controlling root vigor and elongation rate of axile roots in maize. In bean, Liao et al. (2004) identified 16 QTL for root gravitropic traits (8 for shallow basal roots, 5 for relative shallow basal roots and 3 for basal root growth angle), and 6 controlling P uptake under low P conditions. Three of the QTL they found for gravitropic traits were associated with QTL for P uptake under low P, sustaining the idea that root gravitropism contribute to P acquisition. Ochoa et al. (2006) identified two major QTL

controlling adventitious rooting in beans under low P conditions in the field that controlled 61 % of the variation in adventitious roots. Beebe et al. (2006) identified individual QTL controlling basal and tap roots in bean. They found that QTL controlling P accumulation coincided with basal root formation. These results suggest that basal roots are important for P acquisition. The control of root traits by QTL demonstrate that root traits are genetically controlled, therefore, root phenes could be targeted for crop improvement in breeding programs for edaphic stresses. Root traits conferring P efficiency such as long and dense root hairs and root shallowness could be transferred into adapted varieties throughout crosses in traditional breeding methods. An alternative for phenotypic screening would be the use of Marker Assisted Selection (MAS).

Thus, to improve bean yield in low input agrosystems without the addition of fertilizers, breeders need to identify and select genotypes with root systems suitable to the target region. The appropriate approach for variety improvement, particularly in developing countries of Africa and Latin America where the use of molecular technologies for breeding is limited, could be selection through root phenotyping.

The objectives of this research were:

1. To develop a rapid and simple method to evaluate root traits of common bean in the field – *Shovelomics*

2. To assess the genetic diversity of root traits of common bean (*Phaseolus vulgaris* L.) from Andean and Mesoamerican gene pools

3. To estimate the heritability of root hair traits of common bean

Each of these objectives was assessed in the following three individual chapters.

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Chapter 2

Shovelomics: high - throughput phenotyping of common bean root architecture in the field

Abstract

Low phosphorus availability and drought are major constraints to common bean *(Phaseolus vulgaris L.)* production in many developing countries. Genotypic adaptation to drought and low soil P availability is associated with phenotypic variation in root architecture. Bean genotypes with shallow basal root growth angles, several basal root whorls and adventitious roots have advantages in acquiring P, while genotypes with steep basal root growth angles have superior water acquisition under drought. Root systems are difficult to evaluate directly in the field and most root studies are conducted in controlled environments. The objective of this study was to develop a simple method to evaluate root traits of common bean in the field. Thirty genotypes were evaluated in the laboratory and in two field sites in Mozambique in 2008 and 2009. To compare measured and scored values, twenty genotypes were planted in the USA in 2010. Four plants per plot were excavated 45 days after planting, and a 1 to 9 visual scale was used to score 12 root architectural phenes. Significant differences among genotypes within environment and within year were detected for adventitious root number, length and branching, basal root growth angle, basal root whorl number, basal root length and branching, and primary root length and branching. The visual scoring method we developed in the present study separated the root phenes evaluated into 2 to 6 different categories. Laboratory and field results for basal root whorl number and basal root number were consistent. A positive correlation was observed between basal root whorl number measured in the laboratory and basal root whorl number evaluated in the field (R^2 = 0.803). The environment and year did not affect the ranking scores of most of the root traits. Correlation between measured versus scored traits was high and significant (p-value < 0.001) for basal root angle (R^2 = 0.755), adventitious root length (R^2 = 0.733), primary root length (R^2 = 0.644), basal root length (R^2 = 0.584), primary root branching (R^2 = 0.577), and adventitious root branching (R^2 = 0.574). This result indicates that the visually scored traits were good estimators of the measured traits. On average, 2 min were required to evaluate 12 traits in one root crown. Our results indicate that the environment and soil type did

not influence the results of the evaluations. Thus, results obtained with visual scoring could be compared across years and regions. We have developed and validated a visual method for rapid evaluation of common bean root architectural phenes directly in the field. This method can be modified for phenotyping root systems of other dicotyledonous crops. Field phenotyping using shovelomics should have utility for bean breeding for low phosphorus and drought tolerance in developing countries of Africa and Latin America.

Introduction

Low phosphorus (P) availability is a primary constraint to common bean *(Phaseolus vulgaris L.)* production in many developing countries (Lynch, 2007). Root architecture is an important factor for P acquisition (Lynch, 1995 and 1997), and it varies substantially within species, even among closely related genotypes (Lynch, 2005; Lynch and Brown, 2008). Considerable genetic diversity exists within bean for root architectural phenes that is related to growth in low P environments (Lynch and Beebe, 1995; Bonser et al., 1996; Miller et al., 2003; Miguel, 2004; Ochoa et al., 2006; Rubio et al., 2003). Bean genotypes with shallow basal root growth angle, several basal root whorls, and adventitious roots have advantages in acquiring phosphorus under P stress, whereas genotypes with steep basal root growth angles will acquire water in deeper soil horizons (Ho et al., 2004; Lynch, 2005). Greater basal root whorl number increase soil exploitation by increasing the vertical area of root deployment because the upper whorls generate shallow roots and the lower whorls generate roots with steeper angles (Lynch, 2011). Therefore, genotypes with more basal root whorls may also be advantageous in acquiring water under water stress. In order to improve bean yield in low input agroecosystems, breeders need to select genotypes with root systems suitable for the target region.

The root system is often difficult to evaluate directly in the field and most root studies are conducted in controlled environments that do not represent natural conditions. Several methods used to evaluate root systems have been reported. Germination paper (roll ups) and pouches have been used to study root traits in crop seedlings in the laboratory. Zhu and Lynch (2004) used germination paper to study seminal roots, and Zhu et al. (2005a and 2005b) to investigate the genetic basis of root hair and lateral roots in response to P availability. Germination paper was also used to characterize whorls and basal root number (Widrig, 2005), and root hair traits (Vieira, 2007) in bean seedlings. In addition, Bonser et al. (1996) used growth pouches, which are germination paper in plastic bags, to study root growth angle in bean in response to low and high P availability. Hund et al. (2009) developed a phenotyping platform for non-destructive measurement of root growth in maize seedlings using pouches. In addition, Trachsel et al. (2009) used pouches to map quantitative trait loci (QTL) for lateral and axile root growth of maize. Ochoa et al. (2006) used nutrient solution and field studies to investigate the genetic basis of adventitious root formation in beans. Many greenhouse experiments to investigate root systems are conducted in pots or cylinders (mesocosm) filled with media. Liao et al. (2001) used pots to study root gravitropic in response to P availability. Zhu, et al. (2010a) used pots to investigate

root hair length plasticity in maize. Zhu et al. (2010b) used mesocosms to investigate root cortical aerenchyma. Several other greenhouse studies to investigate root traits were conducted in pots (Gahoonia et al., 2005; Ho et al., 2005; Burton, 2010). Systems that use roll ups, pouches, hydroponics or pots are suitable to evaluate large number of genotypes; however, they do not represent the natural soil conditions that roots and plants are exposed to in the field.

Roots have been evaluated in the field. Trachsel et al. (2010) developed a method to visually score 10 architectural root traits of maize crown in 2 minutes. The traits evaluated included: number of whorls occupied by brace roots, number of brace roots, arms of the brace roots originating from whorl 1, whorl 2, number and branching of brace roots, angle and branching of crown roots. Field evaluations of root hair traits of beans (Miguel, 2004; Zhu et al., 2010a), and architectural and anatomical root phenes in maize (Burton, 2010) were also reported. The scoring system developed by Trachsel et al. (2010) for maize is not directly applicable to dicotyledonous crops such as common bean because of significant differences between the root architectures of monocotyledonous and dicotyledonous plants. In maize the main axial roots are the primary root, seminal roots, and nodal roots appearing in successive whorls from the shoot nodes (Hochholdinger and Tuberosa, 2009), whereas in beans the main axial roots consist of the primary root, adventitious roots arising from subterranean hypocotyl tissue, and basal roots arising from the base of the hypocotyl (Zobel, 1996; Basu et al., 2007). Secondary growth of roots occurs in bean but maize, which over time results in bean having large diameter lateral roots with multiple orders of branching (Postma and Lynch, 2011).

The objectives of this study were to develop a rapid method to evaluate root traits of common bean directly in the field; to compare results of root traits obtained in controlled environments with field data, and to compare visual scores with measured root trait values. We anticipate that a validated scoring system for common bean root architecture will have broad applicability for other annual dicotyledonous plants.

Material and Methods

Plant material

Eighty-five common bean genotypes obtained from CIAT were used in this study (Appendixes 2-1, 2-2 and 2-3). Genotypes were chosen based on variation in root traits, and tolerance to low P and drought conditions. G 19833 and DOR 364 were included as check genotypes. G 19833 is an Andean genotype tolerant to low P (CIAT, 1996; Beebe et al., 1997) and it has shallow basal roots (Lynch, 1995; Liao et al., 2001), three basal root whorls (Basu et al., 2007) and several adventitious roots (Ochoa et al., 2006). DOR 364 from Mesoamerican gene pool has poor yield under P deficiency (Beebe et al., 1997), deeper basal roots (Liao et al., 2001), two basal root whorls (Basu et al., 2007). Five genotypes obtained in the Agricultural Research Institute of Mozambique (IIAM): Doutor, LIC-04-1-3, Diacol Calima, Ica Pijão and one commercial variety, Bonus, were included in the experiments. The twenty accessions evaluated in Rock Springs were a subset of the CIAT bean core collection composed of accessions from different races and geographic regions (Colombia, El Salvador, Guatemala, Mexico, Ecuador, Peru, Brazil and Haiti) (Beebe et al., 2000) (Appendix 2-3).

Laboratory experiment

Sixty-four genotypes (Appendix 2-1) were planted in a randomized complete block design (RCBD) in the laboratory in 2006 at Pennsylvania State University (PSU), USA. The experiment consisted of 4 replications over time, and each experimental unit was composed of one plant.

Seeds were surface-sterilized for 1-2 minutes with 10% NaOCl, rinsed with deionized water, mechanically scarified with a razor and germinated in rolls of brown germination paper No 78 (Anchor Paper Company, St. Paul, MN, USA). The rolls were placed upright in 5 liter beakers containing 1 L of 0.5 mM $CaSO_4$. Seeds were allowed to germinate in darkness at 28 ^oC for 3-4 days. The seedlings were then placed in a plant culture room at 26 ^oC for 4 days with 12 hours of light. Basal root whorl number and total number of basal roots were counted 8 days after planting. The roots were stored in 25% ethanol for other analyses.

Field experiment

Field trials were conducted at the IIAM Agriculture Research Station of Chokwe, (24° 31′ S; 33° 0′ E, 40 m.a.s.l) in 2008 and 2009, the Agriculture Research Station of Umbeluzi, Mozambique (26° 03’ S; 32° 21’ E, 64 m.a.s.l) in 2008, and the Russell Larson Agricultural Research Station of The Pennsylvania State University in Rock Springs, Pennsylvania, USA (40^{o} 44’ N; 77^{o} 53’ W, 366 m.a.s.l.) in 2010. The soil at the field site in Chokwe is a Mollic Ustifluvent with silt-loam texture (Mollic Fluvisols, FAO, 1988), while the soil at the Umbeluzi site is a Mollic Ustifluvent with sandy-loam texture (Eutric Fluvisols, FAO, 1988). The P availability at the field where the trials were conducted in Chokwe was 38 ppm (P – Olsen), with pH of 6.8 and 1.8% of organic matter, and in Umbeluzi the P availability was 20 ppm (P – Olsen). In Rock Springs, the genotypes were grown in a Hagerstown silt loam soil (fine, mixed, semi-active, medic Typic Hapludult). The P availability at the field in Rock Springs was 10.5 ppm (P – Mehlich 3 extraction).

Thirty genotypes (Appendix 2-2) were planted in a randomized complete block design (RCBD) in Chokwe in 2008 and 2009, and in Umbeluzi in 2008. The experiment consisted of 4 replications, and each experimental unit was composed of two rows of 5 m. Twenty-five seeds were sown in each row with spacing of 0.7 m between rows and 0.2 m between plants in a row. Nitrogen in the form of urea was applied 25 days after planting at a rate of 30 kg/ha in trials conducted in Chokwe and Umbeluzi. Phosphorus was not applied in all trials. Weed and pest control, and irrigation were applied as needed.

In 2010, a subset of the bean core collection (Appendix 2-3) was evaluated under low phosphorus in Rock Springs in order to compare values of measured and visually scored root traits. The experiment was planted in a RCBD with 4 replications. Seeds of each genotype were sown in one row of 1.6 m, and the space between rows was 0.7 m and between plants in a row was 0.2 m. Each experimental unit had 8 plants. Weed and pest management and irrigation were applied as needed.

Evaluation of root traits

Root crowns of 3 to 4 representative plants per replication were excavated 45 days after planting (DAP), corresponding to the pod filling growth stage R6. The root crowns were excavated 25-30 cm around the shoot with a depth of 25-30 cm. The excavated root crowns were carefully shaken to remove excessive soil. The remaining soil was removed by soaking the root crowns in water containing about 0.5% detergent and rinsing with water from a hose at low pressure. The root crowns were only washed at Rock Springs where the soil was silt-loam. The root crowns evaluated in Chokwe and Umbeluzi were not washed because the roots were clean enough for evaluation after removal of excessive soil residues by hand. The following traits were visually evaluated using 1 to 9 scores: adventitious root length; adventitious root branching; basal root length; basal root branching; primary root length; primary root branching; basal root angle; number of nodules; and root rots. Actual counts were taken for the total number of adventitious and basal roots, and basal root whorls (Table 2-1 and Figure 2-1). One representative score was recorded for each root trait per replication.

In the experiment conducted in 2010 in Rock Springs, 20 genotypes were selected at random from 155 genotypes of the bean core collection. The root traits of the excavated crowns were first measured then saved for subsequent evaluation using 1 to 9 visual scores. The length of adventitious, basal and primary roots was measured with a ruler, and basal root angle was measured with a protractor. Root branching (density) was measured by counting the number of lateral roots in a representative segment of 2 cm of adventitious, basal and primary roots. One representative score or measurement was recorded for each root trait per replication.

Statistical analysis

Data were analyzed using Minitab statistical software Minitab Inc., State College, Pennsylvania, USA, and Statistix, version 8 (Analytical Software, Tallahassee, FL, USA). Analyses of variance were performed separately for laboratory and field experiments. Genotype was considered as fixed effect, and year and environment were random for experiments from Chokwe 2008 and 2009, and Umbeluzi 2008. Correlation analyzes were performed to determine relationship among traits, and to compare laboratory versus field results as well visual scores and measured root trait values.

Results

Root traits variability among genotypes

Genotypes differed significantly for basal root whorl number and basal root number in 8 day-old seedlings ($p \leq 0.01$) (Table 2-2). BRWN varied from 1 to 3.75 and the number of basal roots varied from 4 to 13.5 (data not shown). These two traits were positively correlated (Figure 2.6). We found high phenotypic variation for most root phenes evaluated in the field (Figure 2-2 and Figure 2-3). Significant differences among genotypes within environment and within year were detected for adventitious root number and branching, basal root growth angle, BRWN, basal root number, and primary root length (Table 2-3, Appendix 2-4).

Field and laboratory evaluations were highly correlated

BRWN evaluated in 8 day old seedlings in the laboratory was highly correlated with basal root number and BRWN evaluated in 45 day old plants in the field in Chokwe in 2008 (Figure 2-4, 2-5). Basal root number evaluated in the laboratory and in the field were also highly correlated ($R^2 = 0.66$). The total number of basal roots in 8 day old seedlings evaluated in the laboratory was greater than the total number of basal roots evaluated in 45 day old plants in the field (Figure 2-5), suggesting that basal roots are lost over time in the field. BRWN was strongly correlated with basal root number when both were evaluated in the laboratory ($R^2 = 0.949$, ($p \leq 0.01$), or field in Chokwe 2008 ($R^2 = 0.867$, ($p \leq 0.01$) (Figure 2-6).

Ranking of the genotypes for root phenes was consistent across years and environments

To assess the effects of genotype by environment and genotype by year interactions we performed analysis of variance for all 12 phenes. Our results indicate that the environment and year did not affect the ranking of the genotypes. Phene expression for genotypes was stable across years within an environment and across environments except for adventitious root number and root rot infection (Tables 2-3 and 2-4).

Correlation between scored and measured root traits

In order to validate our field visual root scoring method we compared values of measured phenes with values of scored phenes. Analysis of variance detected significant differences among genotypes for all root phenes that were simultaneously measured and visually scored except for primary root branching (Table 2-5). In addition, correlations between measured and visually scored phenes of twenty genotypes evaluated in Rock Springs varied from moderate to high (Table 2-6 and Figure 2-7). High correlation was found for basal root growth angle, adventitious root length, primary root length, and basal root length. All coefficients of determination for measured versus scored traits were statistically significant, indicating that the visual scores were good estimators of the values of the measured phenes (Table 2-7).

Time required for excavation and evaluation

The time for evaluation varied depending on the soil type where the plants were grown. The total time required from excavation to evaluation varied from 4 minutes in soils with sandy-loam texture to 11 minutes in silt-loam soils. The time required for visual evaluation was not influenced by the soil type in all 3 sites, and the evaluation of 12 phenes from one root crown required an average of 2 minutes (Table 2-8). Root crowns evaluated in Rock Springs (Hagerstown soils) required additional time (approximately 8.5 min) for soaking and washing the roots while root crowns evaluated in Mozambique (Alfisols and Entisols soils) were not washed because the roots were clean enough for evaluations after removal of excessive soil residues by hand. Root excavation required 2.5 min at Rock Springs and 2 min in Chokwe and Umbeluzi (Table 2-8).

Discussion

We developed a simple and rapid method for evaluating root architectural phenes of common bean directly in the field. The following 12 phenes were evaluated from one excavated root crown: 1) adventitious root number; 2) adventitious root length; 3) adventitious root branching; 4) basal root whorl number, 5) basal root number; 6) basal root length; 7) basal root

branching; 8) basal root growth angle; 9) primary root length; 10) primary root branching; 11) number of nodules and 12) incidence of root rot infection. The visual rating developed in the present study is robust and differentiated the genotypes based on the evaluated phenes, and the rating of the genotypes was not influenced by the environment. The soil type influenced the time required for root excavations and washing but it did not affect the results of the root evaluations.

Our approach to high-throughput field phenotyping of bean root architecture is patterned after a similar approach to field phenotyping of maize root architecture (Trachsel et al. (2010). Using maize shovelomics they were able to evaluate 10 maize root phenes from one root crown, and their results were also not influenced by the soil type, demonstrating the robustness of the field phenotyping. The scoring method developed for maize by Trachsel et al. (2010) is different from the bean scoring method because of differences in root architectures of maize (monocotyledonous) and beans (dicotyledonous). According to Hochholdinger and Tuberosa (2009), the main axial roots in maize consist of the primary root, seminal roots, and nodal roots appearing in successive whorls from the shoot nodes, whereas in bean the main axial roots consist of the primary root, adventitious roots arising from subterranean hypocotyl tissue, and basal roots arising from the base of the hypocotyl (Zobel, 1996; Basu et al., 2007). In maize, Trachsel et al. (2010) evaluated the following root phenes: number of whorls occupied by brace roots, number of brace roots, arms of the brace roots originating from different whorls, root density of brace roots, number, angle and branching of crown roots, while in common bean the evaluated root phenes included the number, length and branching of adventitious roots, number, length, branching and angle of basal roots, number of basal root whorls, and length and branching of primary root.

Genotypes evaluated in Rock Springs showed much greater variation in adventitious root length and branching, and primary root length than genotypes evaluated in Chokwe and Umbeluzi (Figure 2-2 and 2-3). In Rock Springs we used a subset of the bean core collection representing bean accessions from different geographic regions and races (Appendix 2-3), and the genetic diversity among these accessions is greater when compared to the genotypes used in Chokwe and Umbeluzi that were mostly improved genotypes developed by CIAT (Appendix 2-2). In addition, in Rock Springs the genotypes were evaluated under low P conditions, which stimulate the elongation and proliferation of lateral roots from adventitious roots (Ochoa et al., 2006, Miller et al., 2003). Our results show that the visual scoring method could separate genotypes (Appendix 2-4) with narrow root trait variation as the set of genotypes evaluated in Mozambique as well as

among genotypes with a wider range of variation evaluated in Rock Springs. The visual scoring could be modified to separate small differences in phenes with narrow variation. We found relatively narrow variation among genotypes in root branching and root length. To maximize the variation of root branching we could measure root density by counting the number of lateral roots in a representative segment of the root. Our shovelomics method excavates the root crown 25 cm from the base of the stem and 30 cm deeper, thereby excising some of the adventitious, basal and primary roots. One alternative to minimize root damage is to increase the wide and depth of the root crown, but more area will be needed for field evaluations of a large number of genotypes particularly.

The correlation coefficients between measured and scored phenes were moderate to strong for basal root angle, adventitious root length, primary root length, basal root length, primary root branching, and adventitious root branching, and relatively weak for basal root branching (Table 2-6 and Figure 2-7). These correlations indicate that the visual method developed in this study was accurate for evaluating phenes of bean directly in the field. A relatively weak correlation ($R^2 = 0.312$, $p < 0.05$) between measured and scored basal root branching indicates that the criterion used for visual scores did not give accurate estimation of root density (number of lateral roots in a root segment). Our visual scores were based on root branching including secondary branches while the measured method was based on lateral root density (number of lateral roots in 2 cm). These differences probably influenced the correlation results. We observed that roots with high lateral root density (number of laterals in 2 cm) did not consistently have multiple branches of secondary or higher orders. Although the correlation between measured and scored values was weak for root branching, the ranking of the genotypes across environments and across years did not change as demonstrated by the lack of interaction genotype by environment and genotypes by year for root branching on basal and primary root classes. This result indicates that the visual score was robust enough to differentiate genotypes based on evaluated phenes. We suggest a modification of the method for evaluation of root branching to improve accuracy. Instead of scoring root branching by evaluating the presence of multiple branches of different orders, we could estimate the root branching by directly counting the number of lateral roots in a representative segment of the root.

Root architecture is an important factor for determining acquisition of resources in the soil (Lynch and Brown, 2001; Lynch, 2007), and variation in root phenes of bean have been reported. In beans, deeper roots have been associated with drought tolerance, while shallow roots

are associated with topsoil exploration of immobile nutrients such as P (Lynch and Brown, 2001; Ho at al., 2005; Lynch, 2005). Therefore, the visual method we developed in this study could be used to identify, select and develop genotypes with root phenes suitable for regions with low P or drought stress. In this study we were able to identify genotypes with root phenes suitable for low P conditions.

The importance of basal roots for P acquisition has been reported (Lynch, 2005; Widrig, 2005). Genotypes with more BRWN have potential of having more basal roots and they may perform better in low P conditions since basal roots explore most of the topsoil where P is concentrated. Laboratory and field results of BRWN and basal root number evaluations were consistent. Basal root whorl number evaluated in 8 day old bean seedlings was highly correlated with basal root number evaluated in 45 day old plants in the field ($R^2 = 0.803$). From these results we can conclude that BRWN evaluated in bean seedlings in the laboratory predicts the total number of basal roots of a genotype in the field although we found a reduction in basal roots in the field. The reduction of the number of basal roots in the field may probably be due to insects and mechanical damage of the roots when compared to laboratory evaluations where root seedlings were less exposed to these factors. Based on these results we would recommend the evaluation of BRWN and basal root number in the laboratory using "roll up method" to save time, reducing the number of phenes to be evaluated in the field. Results from laboratory evaluations could then be used to predict the total number of basal roots of a genotype in the field.

Adventitious roots are the shallowest bean root class since they emerge from the subterranean part of the hypocotyl. Several studies reported that adventitious roots enhance P acquisition (Lynch et al., 1995; Lynch and Brown, 2001; Walk, et al., 2006; Ochoa et al., 2006). In the present study the interaction genotype by year was not significant for all root phenes measured in 2008 and 2009 in Chokwe except for adventitious root number. The year affected the number of adventitious roots within environment, probably due to differences in precipitation that influenced the soil moisture in the surface. The total precipitation during the bean growing season was 64.6 mm in 2008 and 109.5 mm in 2009. Probably some adventitious roots dried out in the soil surface in 2008 and consequently reduced the total number of adventitious roots. The lack of interaction genotype by year for other phenes in Chokwe indicates that the year did not affect the ranking of the genotypes within environment.

Moreover, the interaction genotype by environment was not significant for any phenes except for adventitious root number and branching (Table 2-3). The differences in soil temperature and soil moisture probably influenced the proliferation of adventitious roots in the two environments. In Chokwe the experiments were conducted during the cool season (April to June 2008), and in Umbeluzi the experiment was installed from September to November 2008. In this period the temperatures in Umbeluzi were relatively high and may have caused desiccation of adventitious roots due to exposure to high soil temperature and low moisture in the soil surface since adventitious roots are located in the topsoil. Furthermore, the trials conducted in Mozambique were furrow irrigated and irregular accumulation of residual water could result in differences in soil moisture that probably affected the proliferation of adventitious roots in some spots. Factors such as depth of planting can also affect the proliferation of adventitious roots (Rubio and Lynch, 2006). Genotypic variation in adventitious root in bean has been reported (Miller et al., 2003; Ochoa et al., 2006), and adventitious roots have been reported to enhance P acquisition (Lynch and Brown, 2001; Walk et al., 2006; Ochoa et al., 2006). Thus, selection of genotypes with many adventitious roots will improve P uptake in regions with P stresses, provided there is adequate soil moisture near the surface. Two major QTL controlling formation of adventitious rooting in bean have been identified (Ochoa et al., 2006). Thus, an alternative for selection by root phenotyping would be the use of Marker Assisted Selection.

We did not detect significant differences among genotypes in number of nodules and root rot infection in Chokwe and Umbeluzi. The 30 genotypes evaluated in Chokwe and Umbeluzi did not produce many nodules when compared to the 20 genotypes from the bean core collection evaluated under low phosphorus in Rock Springs (data not shown). The soil in Mozambique may not have sufficient Rhizobium inoculum compatible with bean for optimal nodulation. In addition, the application of Nitrogen fertilizer probably reduced the activity of the Rhizobium. Most of the nodules found in genotypes evaluated in Rock Springs were concentrated on adventitious roots. Correlation between number of adventitious roots and number of nodules evaluated in Rock Springs were not significant at 1% (R^2 = 0.004). This result indicates that production of nodules in bean is independent from the number of adventitious roots.

The total time required from excavation to evaluation of one root crown varied from 4 minutes in the African soils to 11 minutes in the silt loam soil of Pennsylvania. Soils with higher proportion of clay like the soil in Rock Springs are heavy and difficult to excavate by hand with a shovel, and the root crowns need to be soaked before washing to minimize root breakage. Root

crowns from soils with medium texture were clean enough for evaluation without root washing. Thus, the visual evaluations using the shovelomics method would require more time for excavation and root washing when the plants are grown in soils with a high proportion of clay than in soils with higher content of sand. Trachsel et al. (2010) took 2 min to evaluate 10 architectural root phenes of maize, and the total time for evaluation varied from 5 min in sand soils to 10 min in relatively heavy soils.

The shovelomics method developed in the present study could be adapted for evaluation of root phenes of other crops. Trachsel et al. (2010) used visual scores to phenotype maize genotypes in the field and they found significant differences in root traits among genotypes. The visual method we present in this study is accurate, cost effective and does not require expensive equipment. This method is appropriate tool for root phenotyping for developing countries of Africa and Latin America with little or no advanced technologies for agriculture research. Drought and low P are the main limitation to bean production in Africa and Latin America (Wortmann and Allen, 1994; Wortmann et al., 1998; CIAT, 2001; Lynch 2007). Therefore, root phenotyping directly in the field using a shovel to excavate the roots would be an appropriate approach for breeding programs in these countries.

The root scoring methods we developed could be improved to maximize differences among genotypes in root phenes that had narrow variation such as root length and branching. For instance, we can increase the width and depth of excavation around the root crown to minimize root damage by the shovel, evaluate the root branching by counting the number of lateral roots in a representative segment of the root (root density). In addition to root rot infection, we can visually evaluate other biotic stresses such as nematode infection. Similarly to the maize shovelomics developed by Trachsel et al. (2010), the bean shovelomics developed here can be modified to phenotype root phenes of other dicotyledonous crops with similar root systems. The root rating method of Trachsel et al. (2010) can directly be used to phenotype root system of graminaceous crops such as sorghum and millet. Thus, the field root phenotyping we developed for bean could be modified for other dicots crops such as cowpea. For cowpea we could exclude basal root whorls and basal root phenes that are not present.

Conclusion

We have developed and validated a visual method for rapid evaluation of the bean root architectural phenes directly in the field. Although we found differences in root phenes across years and environments, they did not lead to genotype by year, and genotype by environment interactions in most phenes. Two minutes were required to evaluate 12 phenes from one bean root crown. The soil type influenced the time required for root excavations and washing but did not affect the results of the evaluations. Thus, results obtained with scoring methods could be compared across years and regions. The moderate to high correlation obtained between lab and field data, and between scored and measured phenes indicate that the field evaluation was good estimator of the root phenes measured in the laboratory, and the scored phenes was good estimator of the measured phenes. The visual score method presented in this study can be modified for phenotyping root system of other crops. The shovelomics method will permit visual identification and selection of root system adapted to regions with problems of drought and low soil fertility.

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Table 2-1. Description of the visual scores on a 1-9 scale used to evaluate root traits in the field. Actual number per plant was recorded for adventitious and basal roots and basal root whorl number. Illustration of the evaluated root phene is in Figure 2-1.

Description	***Corresponding visual score**
Adventitious root number	Actual number per plant
Adventitious root length	1 = ≤ 1 cm to 9 = 15-20cm
Adventitious root branching	1 = no lateral branching to 9 = multiple branches with up to 4 orders of branching
Basal root whorl number	Actual number per plant
Basal root number	Actual number per plant
Basal root length	1 = ≤ 1 cm to 9 = 15-20cm
Basal root branching	1 = no lateral branching to 9 = multiple branches with up to 4 orders of branching
Basal root growth angle	1 = Horizontal to 9 = Vertical (in relation to soil surface)
Primary root length	1 = ≤ 3 cm to 9 = 25-30 cm (depth of excavation)
Primary root branching	1 = no lateral branching to 9 = multiple branches with up to 4 orders of branch
Nodulation	1 = Excellent (>80 nodules per plant) to 9 = < 10 nodules
Root rot infection	1 = no visible symptoms to 9 = 75% or more of the hypocotyl and root with severe lesions

* - Adventitious and basal root length: 1 = ≤ 1cm; 2 = 2-4 cm; 3 = 4-5cm; 4 = 6-7 cm; 5 = 8-9 cm; 6 = 10-11 cm; 7 = 12-13cm; 8 = 14-15 cm; 9 = 15-20 cm or more.

Adventitious, basal and primary root branching: 1 = no lateral branching; 3 = 1 order of ramification; 5 = 2 orders of ramification; 7 = 3 orders of ramification; 9 = multiple branches with up to 4 orders of branching.

Basal root branching: 1 = no lateral branching; 3 = 1 order of ramification; 5 = 2 orders of ramification; 7 = 3 orders of ramification; 9 = multiple branches with up to 4 orders of branching.

Basal root growth angle: 1 = horizontal (10^{o}); 6 = 60^{o}; 9 = Vertical (90^{o}).

Primary root length: 1 = ≤ 3 cm; 2 = 4-6 cm; 3 = 7-9 cm; 4 = 10-12 cm; 5= 13-15 cm; 6 = 16-18 cm; 7 = 19-21 cm; 8 = 22-24 cm; 9 = 25-30 cm.

Nodulation: 1 = Excellent; > 80 pink/red nodules; 3 = Good: 41-80 nodules; 5 = Intermediate: 21-40 nodules; 7 = Poor: 10-20 nodules; 9 = less than 10 nodules.

Root rot: 1 = no visible symptoms; 3 = 10% hypocotyl and root with light lesions; 5 = 25% hypocotyl and root with lesions; 7 = 50% hypocotyl and root with lesions; 9 = 75% or more of hypocotyl and root with severe lesions.

Table 2-2. Analysis of variance: F values and level of significance for basal root number (BRN) and basal root whorl number (BRWN) measured in 8 day-old seedlings in the laboratory. Means of 4 replications and 64 genotypes and the standard errors are presented. *** - Significant at $p < 0.001$.

	F value	
Source of variation	**BRWN**	**BRN**
Genotype	12.75 ***	11.77 ***
	BRWN	**BRN**
Mean	2.3672	8.848
Standard error (SE)	0.0390	0.141

Table 2-3. Analysis of variance of root traits evaluated in two environments (Chokwe and Umbeluzi) and two years (2008 and 2009 in Chokwe) in 30 genotypes. F values and significance levels for the effect of the environment, year and their interactions with genotype are shown for the following traits: adventitious root number (ARN), adventitious root length (ARL), adventitious root branching (ARB), basal root whorl number (BRWN), basal root number (BRN), basal root length (BRL), basal root branching (BRB), basal root angle (Angle), primary root length (PRL), primary root branching (PRB), number of nodules (Nodule) and root rot. Level of significance: *** = significant at 1%, ** = significant at 5%, * - significant at 10%, ns = not significant. G = genotype and E = environment.

	ARN	ARL	ARB	BRWN	BRN	BRL	BRB	Angle	PRL	PRB	Nodule	Root Rot
	F-value											
Environment	169***	9.37***	9.43***	5.8**	1.73ns	18.10***	0.82ns	11.97***	160.66***	5.05**	6.37**	8.96***
Genotype	3.81***	1.35***	0.97ns	124.65***	14.01***	1.54***	1.86**	11.06***	1.63**	2.39***	1.44ns	0.86ns
G*E	1.78**	0.92ns	1.49**	0.16ns	0.86ns	1.27ns	0.61ns	0.56ns	1.06ns	0.73ns	0.71ns	1.72**
Year	6.18**	28.66***	0.15 ns	1.63ns	15.84***	2.02ns	19.85***	0.14 ns	6.32**	21.13***	2.43 ns	1.15 ns
Genotype	1.03***	1.76*	1.99**	55.08***	13.57***	1.73*	2.2**	11.59***	1.32 ns	1.16 ns	1.53 ns	0.7 ns
G*Year	1.69**	0.76 ns	1.04 ns	0.43 ns	1.24 ns	0.91 ns	0.6 ns	0.74 ns	1.38 ns	1.18 ns	0.76 ns	1.56**

Table 2-4. Summary statistics of 12 traits evaluated in two environments (Chokwe and Umbeluzi), and in two years (2008 and 2009) in Chokwe in 30 genotypes: adventitious root number (ARN), adventitious root length (ARL), adventitious root branching (ARB), basal root whorl number (BRWN), basal root number (BRN), basal root length (BRL), basal root branching (BRB), basal root growth angle (BRGA), primary root length (PRL), primary root branching (PRB), number of nodules (Nodule) and root rot. The values of means correspond to visual root scores except for ARN, BRWN and BRN that were actual counts.

Environments: Chokwe and Umbeluzi

Variable	Environment	Mean	StDev	Minimum	Maximum
ARN	Chokwe	23.33	7.94	6.0	43
	Umbeluzi	12.00	5.47	4.0	30
ARL	Chokwe	4.60	1.79	2.0	9.0
	Umbeluzi	5.09	1.19	3.0	8.0
ARB	Chokwe	2.78	0.84	1.0	6.0
	Umbeluzi	3.15	0.82	2.0	5.0
BRWN	Chokwe	2.30	0.60	1.0	4.0
	Umbeluzi	2.26	0.60	1.0	4.0
BRN	Chokwe	7.63	2.03	3.0	14
	Umbeluzi	7.43	2.04	4.0	15
BRL	Chokwe	7.51	1.24	5.0	9.0
	Umbeluzi	6.93	1.12	5.0	9.0
BRB	Chokwe	3.44	0.69	2.0	6.0
	Umbeluzi	3.51	0.74	3.0	7.0
BRGA	Chokwe	4.39	1.64	1.0	8.0
	Umbeluzi	3.22	1.24	1.0	6.0
PRL	Chokwe	6.73	1.15	3.0	9.0
	Umbeluzi	5.06	0.96	3.0	9.0
PRB	Chokwe	4.25	0.92	2.0	7.0
	Umbeluzi	4.03	0.91	2.0	6.0
Nodules	Chokwe	8.84	0.76	3.0	9.0
	Umbeluzi	8.99	0.09	8.0	9.0
Root rot	Chokwe	1.18	0.46	1.0	3.0
	Umbeluzi	1.02	0.13	1.0	2.0

Continued Table 2-4.

Years: 2008 and 2009

Variable	Year	Mean	StDev	Minimum	Maximum
ARN	2008	23.33	7.94	6.0	43
	2009	25.66	7.24	8.0	46
ARL	2008	4.60	1.79	2.0	9.0
	2009	5.40	1.31	2.0	9.0
ARB	2008	2.78	0.84	1.0	6.0
	2009	2.74	0.54	2.0	5.0
BRWN	2008	2.30	0.60	1.0	4.0
	2009	2.33	0.58	1.0	4.0
BRN	2008	7.63	2.03	3.0	14
	2009	8.28	1.87	4.0	16
BRL	2008	7.51	1.24	5.0	9.0
	2009	7.70	1.07	3.0	9.0
BRB	2008	3.44	0.69	2.0	6.0
	2009	3.16	0.42	2.0	4.0
BRGA	2008	4.39	1.64	1.0	8.0
	2009	4.34	1.81	1.0	8.0
PRL	2008	6.73	1.15	3.0	9.0
	2009	7.07	0.76	5.0	9.0
PRB	2008	4.25	0.92	2.0	7.0
	2009	3.76	0.62	2.0	5.0
Nodules	2008	8.84	0.76	3.0	9.0
	2009	8.94	0.29	7.0	9.0
Root rot	2008	1.18	0.46	1.0	3.0
	2009	1.11	0.34	1.0	1.0

Table 2-5. Analysis of variance of measured and scored traits evaluated in 20 bean genotypes in Rock Springs in 2010. The levels of significance among genotypes are presented for measured and scored traits. The measured values were taken for adventitious root length (ARL), adventitious root branching (ARB), basal root length (BRL), basal root branching (BRB), basal root growth angle (BRGA), primary root length (PRL) and primary root branching (PRB). The mean of adventitious root number (ARN), basal root whorl number (BRWN), basal root number (BRN) and number of nodules (Nodul.) correspond to actual counts per plant. The root lengths were measured in cm. The root branching correspond to the number of lateral roots in 2cm, and the BRGA was measured in degree. Means of scored values are presented for ARL, ARB, BRL, BRB, BRGA, PRL, PRB and root rot infection (R rot). Level of significance: *** = significant at 1%; ** = significant at 5%; * = significant at 10%, ns = not significant.

	ARN	BRWN	BRN	ARL	ARB	BRL	BRB	BRGA	PRL	PRB	Nodul.	R rot
Source of variation												
						F value for measured phenes						
Genotype	7.3***	8.7***	7.9***	1.9**	2.8***	2.8***	1.2ns	2.3***	1.9**	1.1ns	3.98***	-
						F value for scored phenes						
Genotype	-	-	-	4.7***	2.2**	2.3***	2.5***	6.0***	2.5***	1.2ns	-	1.2ns

Table 2-6. Coefficients of determination (R^2) between measured and scored traits evaluated in 20 genotypes in Rock Springs and their respective levels of significance. ARL = adventitious root length, ARB = adventitious root branching, BRL = basal root length, BRB = basal root branching, Angle = basal root angle, PRL = primary root length and PRB = primary root branching. Level of significance: *** = significant at 1%; ** = significant at 5%.

Phene	ARL	ARB	BRL	BRB	Angle	PRL	PRB
R^2	0.733	0.574	0.584	0.312	0.755	0.644	0.577
P-value	***	***	***	**	***	***	***

Table 2-7. Summary statistics for measured and scored traits evaluated in 20 genotypes in Rock Springs, 2010. Adventitious root length (ARL), adventitious root branching (ARB), basal root length (BRL), basal root branching (BRB), basal root growth angle (BRGA), primary root length (PRL) and primary root branching (PRB). The mean of adventitious root number (ARN), basal root whorl number (BRWN), basal root number (BRN) and number of nodules correspond to actual counts per plant. The root branching (density) correspond to the number of lateral roots in 2cm, and the angle was measured in degrees. Means of scored values are presented for ARL, ARB, BRL, BRB, Angle, PRL, PRB and root rot infection.

Measured values

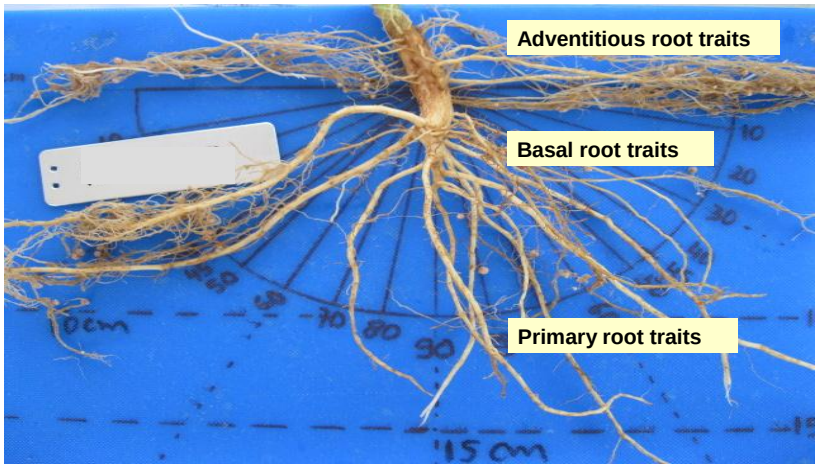
Variable	Mean	StDev	Minimum	Maximum
ARN	19.66	9.50	8.0	54.0
ARL	14.30	5.55	4.0	35.0
ARB	6.19	2.49	2.0	14.0
BRWN	2.20	0.64	1.0	3.0
BRN	8.24	2.24	4.0	12.0
BRL	22.51	5.28	10.0	37.0
BRB	6.51	1.89	3.0	12.0
BRGA	45.44	17.60	10.0	80.0
PRL	17.51	4.75	8.0	30.0
PRB	7.51	1.86	4.0	12.0
N.Nodules	31.20	27.85	0.0	130.0

Scored values

Variable	Mean	StDev	Minimum	Maximum
ARL	6.30	1.88	3.0	9.0
ARB	3.51	1.37	1.0	8.0
BRL	8.16	0.93	5.0	9.0
BRB	3.68	1.32	2.0	7.0
BRGA	4.79	2.17	1.0	9.0
PRL	6.48	1.66	3.0	9.0
PRB	4.14	1.21	2.0	7.0
Root rot	1.40	0.84	1.0	5.0

Table 2-8. Time required for field evaluation of 12 root phenes from one crown in different soil textures: Chokwe: Mollic Ustifluvent (silt-loam texture), Umbeluzi, Mollic Ustifluvents (sandy-loam texture) and Rock Springs Typic Hapludalf (silt-loam texture).
- = Root crowns were not washed

Activity	Mollic Ustifluvents (Chokwe)	Mollic Ustifluvents (Umbeluzi)	Typic Hapludalf (Rock Springs)
Crown excavation	2.0 min	2.0 min	2.5 min
Soaking	-	-	5.0 min
Washing	-	-	1.5 min
Evaluation	2.0 min	2.0 min	2.0 min
Total	4.0 min	4.0 min	11 min



A) Bean root crown illustrating root traits evaluated in the field

Root trait	ARL	ARB	BRL	BRB	PRL	PRB
Represent. root						
Score	2, 3, 5, 7, 9	1, 3, 5, 7, 9	3, 5, 7, 9	3, 5, 7, 9	1, 3, 5, 7, 9	2, 3, 5, 7

B) Representative root scores

Score	1	3	5	7	9
Represent. BRGA					

C) Representative scores of basal root grow angle (BRGA)

Figure 2-1. A) Common bean root crown with root traits evaluated in the field; B) representative root scores; C) representative BRGA. Adventitious root length (ARL) and branching (ARB); Basal root whorl number (BRWN), basal root length (BRL), branching (BRB), and growth angle (BRGA); Primary root length (PRL) and branching (PRB). Other traits included the number of adventitious and basal roots, number of nodules and root rot infection.

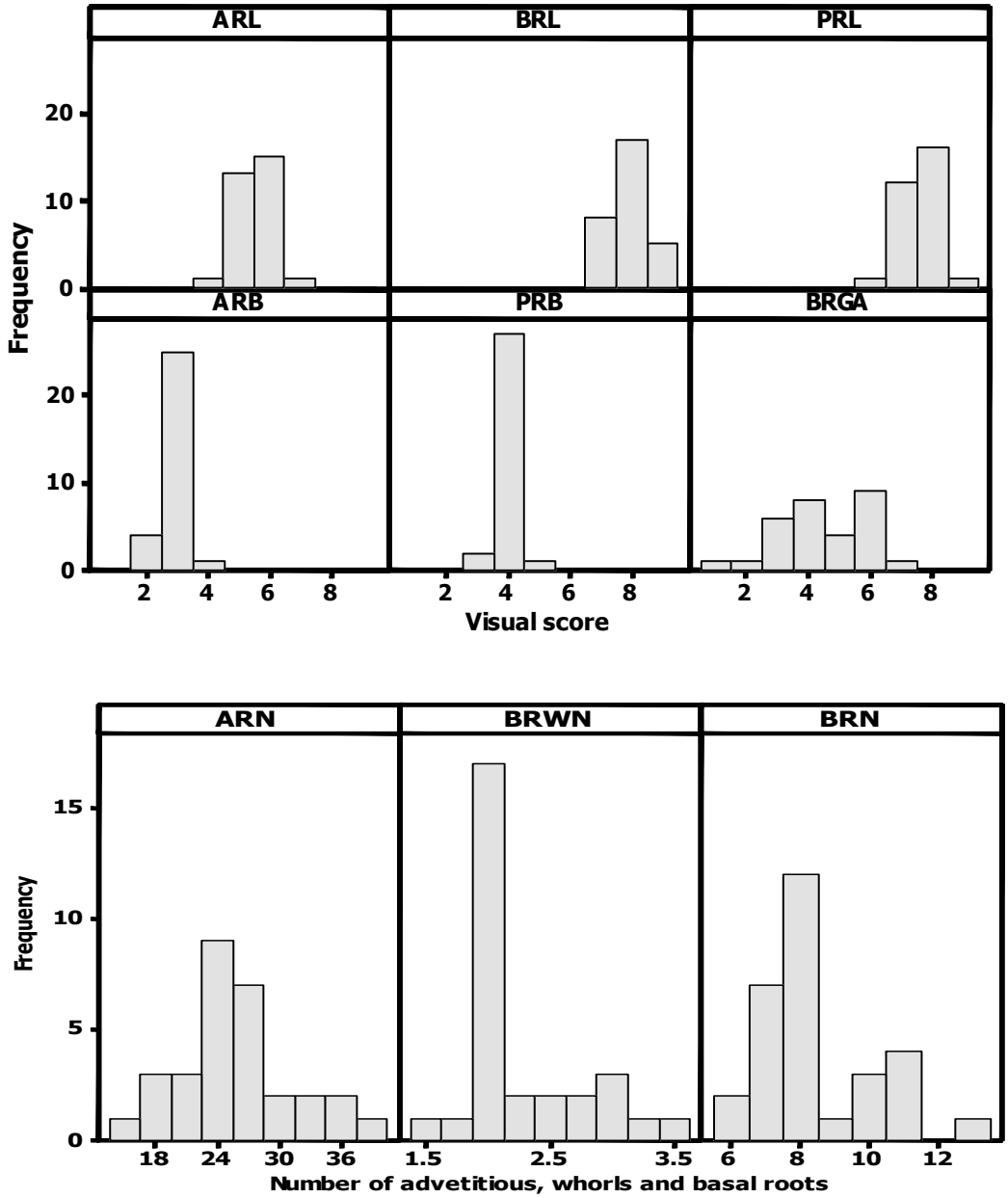


Figure 2-2. Frequency distribution of 30 genotypes evaluated in the field in Chokwe 2009. ARL = adventitious root length; BRL = basal root length; PRL = primary root length; ARB = adventitious root branching; PRB = primary root branching; BRGA = Basal root growth angle ARN = Adventitious root number; BRWN = Basal root whorl number, BRN = Basal root number. The values of ARL, BRL, PRL, ARL, PRB and BRGA correspond to visual scores, and ARN, BRN and BRWN are actual number per plant. The data are average of 4 replications.

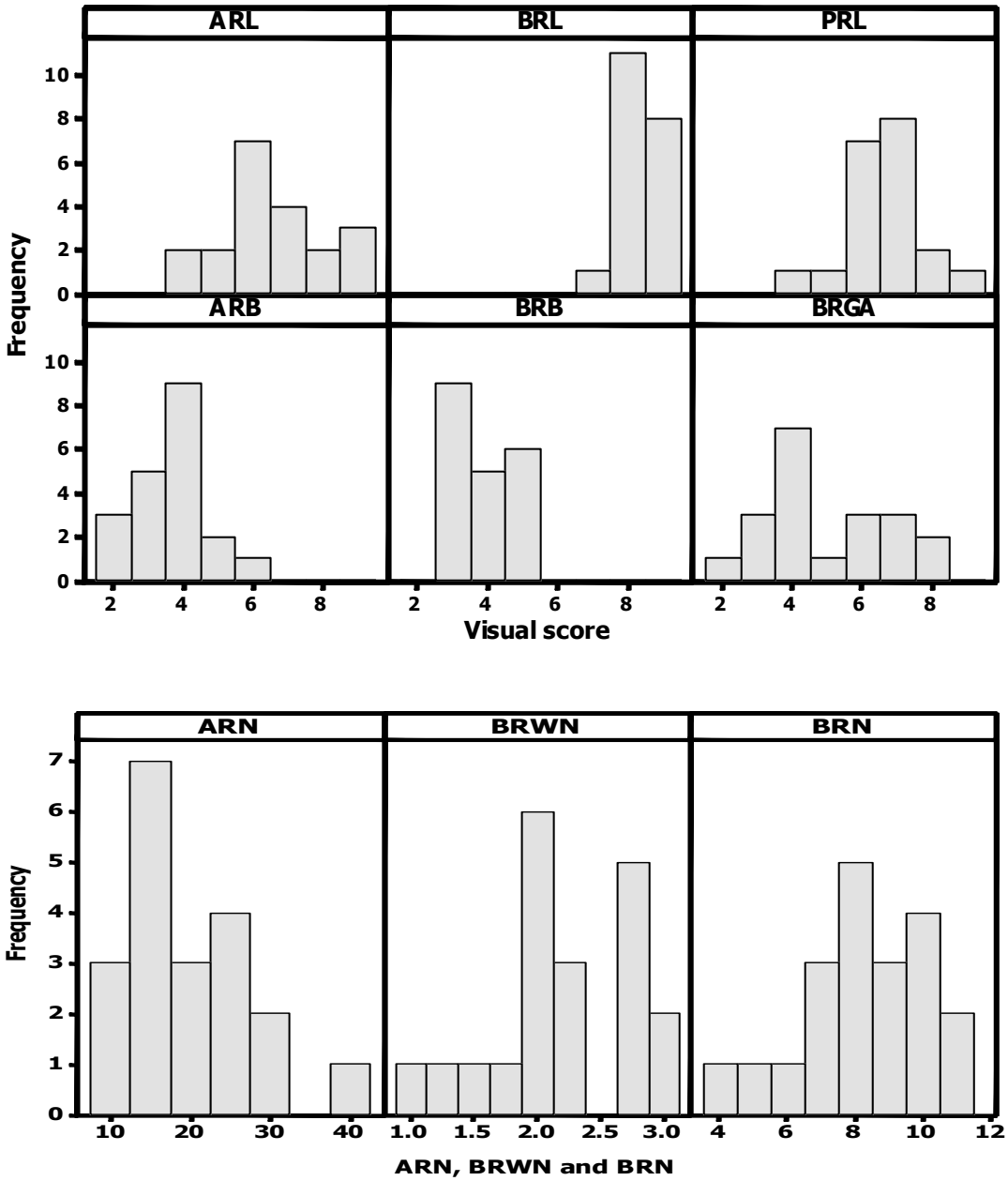


Figure 2-3. Frequency distribution of 20 genotypes evaluated in the field in Rock Springs under low phosphorus conditions. ARL = adventitious root length; BRL = basal root length; PRL = primary root length; ARB = adventitious root branching; BRB = basal root branching; PRB = primary root branching; ARN = Adventitious root number; BRN = Basal root number; BRWN = Basal root whorl number, BRGA = Basal root growth angle. The values for ARL, BRL, PRL, ARL, BRB and BRGA are visual scores. ARN, BRN and BRWN are actual number per plant. The data are average of 4 replications.

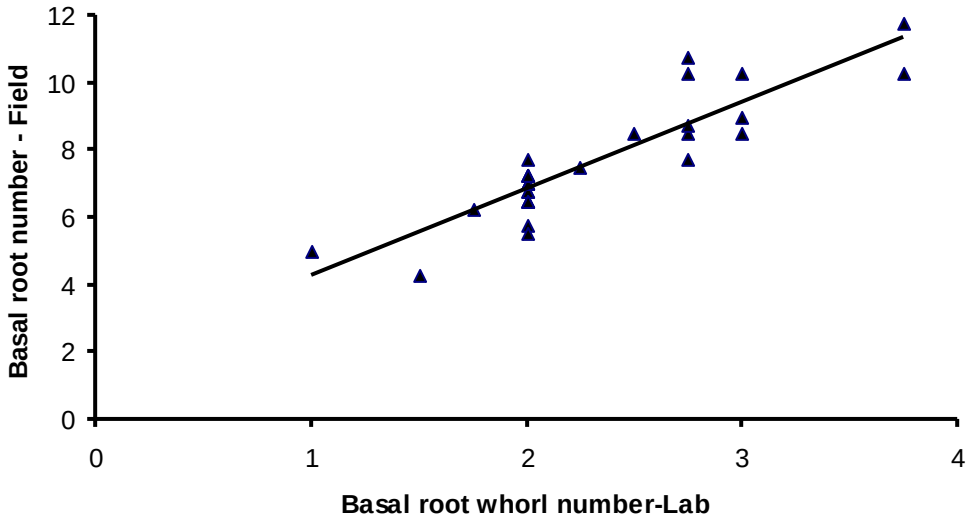
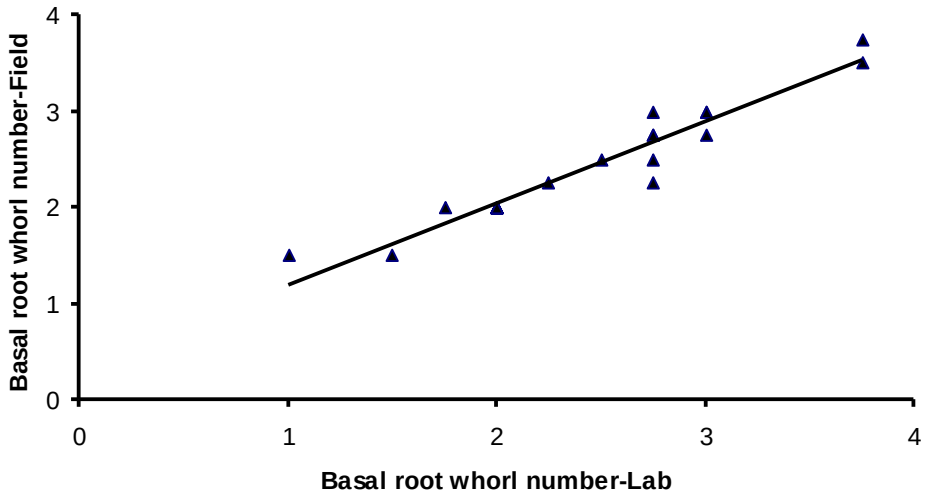
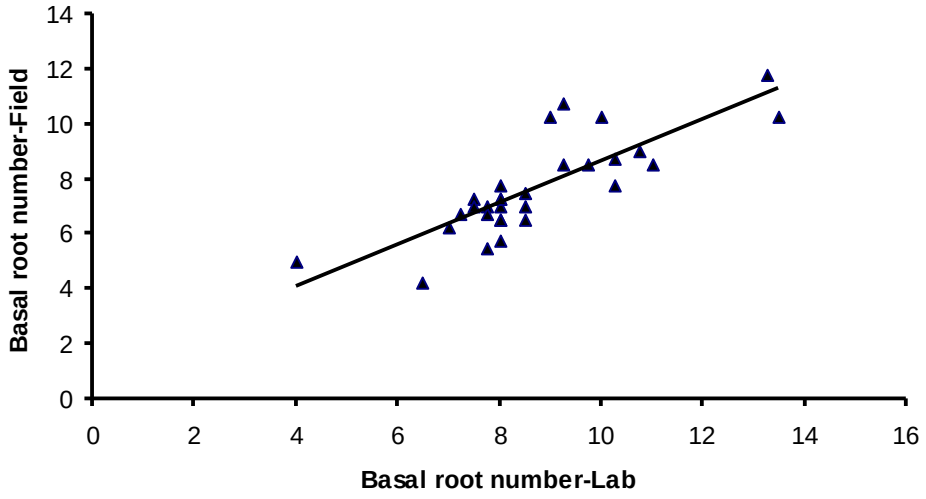


Figure 2-4. Correlation between basal root whorl number evaluated in the laboratory (8 DAP) and basal root number evaluated in the field at 45 DAP. ($R^2 = 0.803$, $p < 0.001$). Laboratory screening of basal root whorl number predicted the number of basal roots of the genotypes in the field. Each point represents an average of 4 replications of 30 bean genotypes.

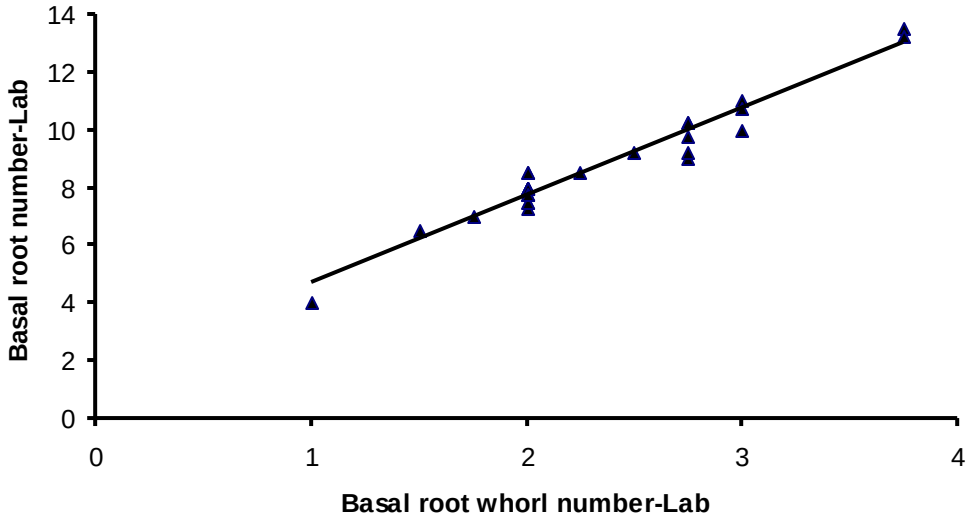


a) $R^2 = 0.93$, $p < 0.001$

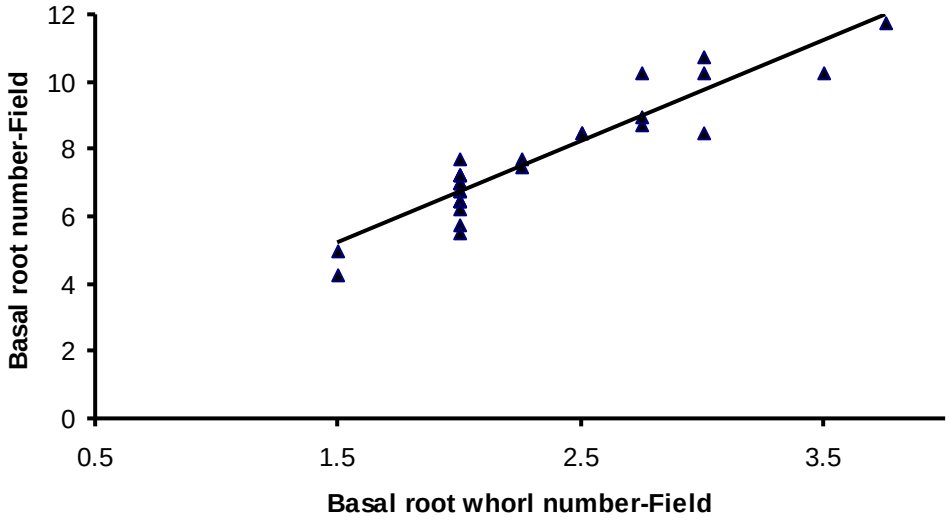


b) $R^2 = 0.66$, $p < 0.001$

Figure 2-5. Correlations between BRWN evaluated in the laboratory and BRWN evaluated in the field in Chokwe, 2009 a); and total number of basal roots evaluated in the lab and total number of basal roots evaluated in the field b). Graph (b) shows that most of the genotypes had reduced number of basal roots in the field. Each point represents an average of 4 replications of 30 bean genotypes.



a) Laboratory. $R^2 = 0.949$, $p < 0.001$



b) Field. $R^2 = 0.867$, $P < 0.001$

Figure 2-6. Scatterplots showing high correlations between basal root whorl number and total number of basal root both in the laboratory a) and field b). Each point represents an average of 4 replications of 30 bean genotypes.

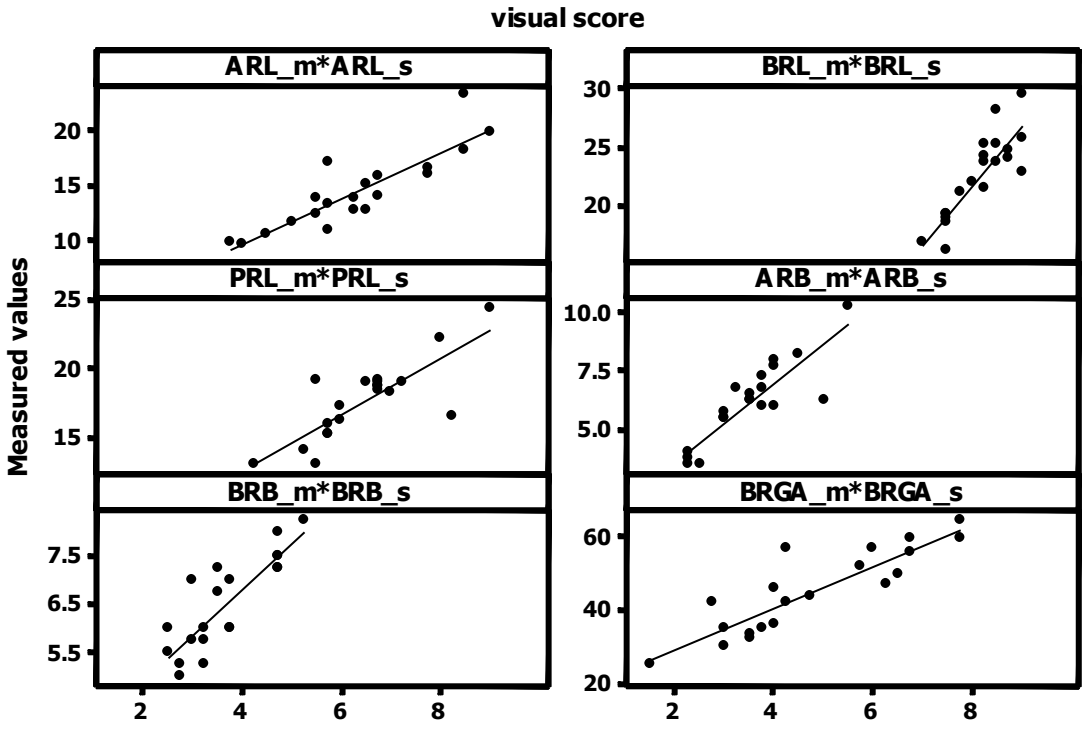


Figure 2-7. Scatterplot showing relationship between measured and scored values in plants evaluated 45 days after planting. Each point represents an average of 4 replications of 20 genotypes selected from the bean core collection. The coefficients of determination (R^2) for each root phene are presented in Table 2-6. ARL = Adventitious root length, ARB = Adventitious root branching, BRL = Basal root length, BRB = Basal root branching, Angle = Basal root angle and, PRL = Primary root length. m = measured value and s = scored value. The root traits were measured in cm for length, degree for BRGA, and actual number of lateral roots in 2 cm for root branching. The 1 – 9 visual scores are described in Table 2-1.

Chapter 3

Diversity of root traits of common bean (*Phaseolus vulgaris* L.) from Andean and Mesoamerican gene pools

Abstract

Low phosphorus availability and drought are major constraints to common bean *(Phaseolus vulgaris L.)* production in many developing countries. The root system is an important factor for plant productivity. Plants evolved a wide range of adaptations to enhance phosphorus (P) and water acquisition from the soil. Bean genotypes with shallow roots, several basal root whorls, adventitious and basal roots have advantages in acquiring P from low P soils, while genotypes with deeper basal roots and longer primary roots will acquire water from deeper soil horizons. Variation in root traits has been reported in many crops. Information on diversity of root traits is crucial for development of genotypes adapted to a specific environment. To assess the diversity of root phenes in beans, 165 accessions from the bean core collection from CIAT were planted in the laboratory and field in 2010 in Pennsylvania, USA. Fifteen root phenes were evaluated from one root crown: adventitious root number, length, branching and diameter, basal root number, length, branching and diameter, basal root growth angle, primary root length, branching and diameter, basal root whorl number, number of nodules, and root rot infection. Substantial phenotypic variation in root traits among genotypes was found in adventitious, basal and primary root traits. Variation among genotypes within gene pools, genotypes within race, and genotypes within country of origin were significant for all 15 root phenes. Accessions from Andean races had a greater number of whorls and basal roots, more lateral branches on basal and primary roots, and shallower basal roots than accessions from the Mesoamerican gene pool. Mesoamerican accessions had long and dense root hairs, many adventitious roots and deep basal roots. Tradeoffs between adventitious root number and basal root number and between adventitious root number and BRWN were detected, indicating the existence of mechanisms of root compensation. Principal component analyses revealed that most of the total variation within Andean and Mesoamerican gene pools was associated with adventitious and basal root classes and a small proportion of variation was associated with primary root traits. Genotypes with root

traits associated with adaptation to low P availability were found in both gene pools, and traits associated with adaptation to drought stress were mostly evident in the Mesoamerican gene pool, although some Andean genotypes expressed extensive lateral branches on basal and primary roots that may improve water acquisition from deeper soil horizons. Andean accessions have root traits that are suitable for regions with low P availability that is associated with volcanic soils of the Andes regions, while most of the Mesoamerican accessions were from races Mesoamerica (M1) reported to be tolerant to drought stress and low soil fertility and Durango that are common in semi-arid regions of Central America. Useful root traits for breeding for edaphic stresses were identified in both Andean and Mesoamerican gene pools, which have contrasting root architectural strategies for soil exploration. Breeding for multiple root traits could enhance acquisition of multiple soil resources, particularly in developing countries.

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the priority legume crop in Africa and Latina America (FAOSTAT, 2011; CGIAR, 2012). It is an important grain legume for direct human consumption in the world (CIAT, 1993; CIAT, 2001, Wortmann et al., 1998; Broughton et al., 2003). Morphological and biochemical traits divide beans into two geographically distinct gene pools, Andean and Mesoamerican, which correspond to the centers of bean domestication (Gepts, 1988; Pachico, 1989; Gepts and Debouck, 1991; Singh et al., 1991a; Singh et al., 1991b; Singh et al., 1991c; Noradi et al., 1992; Broughton et al., 2003; Zizumbo-Villarreal et al., 2005). The two gene pools reflect multiple events of domestication within distinct wild populations (Gepts and Bliss, 1986; Beebe et al., 2001). Mesoamerican genotypes predominate in Mexico, Central America, and Brazil, which account for approximately 84% of the production in Latin America (Beebe et al., 2000). Andean genotypes are found in Colombia, Ecuador, Peru, Bolivia and Argentina (Tohme et al., 1996; Beebe et al., 2001). Andean beans are also cultivated in Brazil, Mexico and the Caribbean, as well as temperate climates of North America and Europe (Beebe et al., 2001).

Cultivated beans are subdivided into races based on morphological and ecological adaptation. Mesoamerican beans are classified into four races: Mesoamerican, Durango, Jalisco and Guatemala (Singh et al., 1991a, Beebe et al., 2000 and 2001), and Andean beans are subdivided into three races: Nueva Granada, Peru and Chile (Singh et al., 1991a; Beebe et al., 2001). The average yield of bean is about 800 Kg/ha in Latin America, and 600 Kg/ha in developing African countries (Lynch 2007); these values are below the average bean yield potential that is estimated as 5800 Kg/ha (Lynch, 2007).

Low soil fertility and drought are the primary constraints to common bean production in many developing countries, affecting more than 80% of the global production (Wortmann and Allen, 1994; Lynch, 1997; Wortmann et al., 1998; CIAT, 2001; Lynch 2007). More than 50% of the bean production zones in Africa and Latin America have serious soil fertility problems (Lynch, 2007). Plant adaptation and productivity in a particular environment is primarily

determined by the ability of the species to obtain resources. Root architecture is an important factor in determining acquisition of soil resources (Lynch, 1997; Lynch and Brown, 2001; Lynch, 2007). Thus, genetic variation in root architecture among and between species is related to adaptation to specific environments (Lynch, 2005). Adaptation to low P environments is associated with root traits that enhance topsoil exploration, including shallow basal root growth angles (Bonser et al., 1996; Lynch and Brown 2001; Rubio et al., 2003; Ho et al., 2004; Zhu et al., 2005b), adventitious rooting (Miller et al., 2003; Ochoa et al., 2006), lateral rooting (Zhu et al., 2005a) and elongation (Borch et al., 1999), root hair length and density (Bates and Lynch, 1996; 2000; and 2001; Gahoonia et al., 1997; Miguel, 2004), aerenchyma formation (Fan et al., 2003) and reduced root respiration (Nielsen et al., 2001). Drought stress is also related to root system distribution in the soil (Sarker et al. 2005). Root architectures with deeper and extensive roots will explore deeper soil horizons where water and mobile nutrient are available. In bean for example, drought tolerance has been associated with deeper roots, while acquisition of P has been associated with better topsoil foraging (Lynch and Brown, 2001; Ho et al., 2005; Lynch, 2005). Complementary development of different root classes will be important for acquisition of multiple soil resources. Identification of genotypes with root phenes adapted to low P availability and drought stresses would be useful for crop improvement.

Interest in root architecture as a criterion for selection for crop adaptation to edaphic stresses has increased (Lynch and Brown, 2001; Vance et al., 2003; Lynch, 2007; Manschadi et al., 2008; Ramaekers et al., 2010; Coudert et al., 2010). Genetic variability in root traits among genotypes in different crops has been reported (Bonser et al., 1996; Gahoonia et al., 1997 and 2005; Miller et al., 2003; Rubio et al., 2003; Zhu and Lynch 2004; Zhu et al., 2005a and 2006; Burton, 2010; Bayuelo-Jiménez et al., 2010). In maize, variation was reported for root architectural traits (Trachsel et al., 2010; Burton, 2010) and for root anatomical traits (Burton, 2010). Sarker et al. (2005) reported variation in taproot length and number of lateral roots in lentil. In beans, variation was reported for basal root whorl and basal root number (Widrig (2005), for root hair length and density (Miguel 2004, Vieira et al. 2007), and for adventitious root number in the field (Ochoa et al. 2006). Considering the large diversity of root traits, incorporation of root phenes into plant breeding programs would be useful for crop improvement.

Quantitative Trait Loci (QTL) controlling root traits in crops have also been reported. In roots, QTL have been identified in maize for root hair length associated with low and high P (Zhu

et al., 2005c), lateral rooting (Zhu et al., 2005a), seminal root length associated with low and high P (Zhu et al., 2006). Trachsel et al. (2009) identified QTL controlling root vigor and elongation rate of axile roots in maize. In bean, Liao et al., (2004) identified 16 QTL for root gravitropic traits (8 for shallow basal root length, 5 for relative shallow basal root length and 3 for basal root growth angle) and 6 controlling P uptake under low P conditions. Three of the QTL for gravitropic traits were associated with QTL for P uptake under low P, supporting the idea that root gravitropism contributes to P acquisition. Ochoa et al. (2006) identified two major QTL controlling adventitious rooting in bean under low P conditions in the field that controlled 61 % of the variation in adventitious roots. Beebe et al. (2006) identified individual QTL controlling basal and tap roots. Their results showed that QTL controlling P accumulation coincided with basal root formation. These results suggest that basal roots are importance for P acquisition. The control of root traits by QTL demonstrate that root traits are genetically controlled, therefore, root traits could be targeted for crop improvement in breeding programs for development of varieties adapted to specific edaphic stresses. Since root traits are genetically controlled, Marker Assisted Selection (MAS) could be used as an alternative for phenotypic root screening.

Substantial genetic diversity exists within common bean for adaptation to low P environments (Lynch and Beebe, 1995; Miller et al., 2003; Ochoa, et al., 2006; Lynch 2011). However, information about diversity of common bean root systems in the field is lacking. Information on diversity of root phenes is necessary in breeding programs for identification and development of bean cultivars tolerant to abiotic stresses. Our hypothesis is that root traits will vary substantially between gene pools and within gene pools or geographic regions. The objectives of the present study were to assess the phenotypic diversity of root phenes in Andean and Mesoamerican gene pools, to identify sources of useful root phenes from Andean and Mesoamerican gene pools, and to identify genotypes with root traits suitable for low P soils and drought stress.

Material and Methods

Plant material

One hundred ninety six (196) genotypes selected from the common bean core collection from CIAT (International Center for Tropical Agriculture) were used for laboratory and field experiments. The bean accessions selected for field experiment and their respective gene pool, race, country of origin and growth habit are presented in Appendix 3-1. The list includes bean accessions from the Andean and Mesoamerica gene pools, and 8 bean races from 15 different geographic regions. Genotypes G 19833 and DOR 364 were included as checks. G 19833 is an Andean genotype tolerant to low P conditions (CIAT, 1996; Beebe et al., 1997); it has shallow basal roots (Lynch, 1995; Liao et al., 2001), three basal root whorls (Basu et al., 2007) and multiple adventitious roots (Ochoa et al., 2006). DOR 364 from Mesoamerican gene pool has poor yield under P deficiency (Beebe et al., 1997), deeper basal roots (Liao et al., 2001), and two basal root whorls (Basu et al., 2007).

Laboratory experiment

One hundred sixty five (165) genotypes were planted in a randomized complete block design (RCBD) in the laboratory in 2010 at The Pennsylvania State University (PSU), USA. The experiment consisted of 4 replications over time, and each experimental unit was composed of one plant.

Seeds were surface-sterilized for 1-2 minutes with 10% NaOCl, rinsed with deionized water, mechanically scarified with a razor and germinated in rolls of brown germination paper No 78 (Anchor Paper Company, St. Paul, MN, USA). The rolls were placed upright in 5 liter beakers containing 1 L of 0.5 mM $CaSO_4$. Seeds were allowed to germinate in darkness at 28 ^oC for 3-4 days. The seedlings were then placed in a plant culture room at 26 ^oC for 4 days with 12 hours of light. Basal root whorl number and total number of basal roots were counted 8 days after planting. Roots were separated from the shoots and stored in 50% ethanol for analysis of root hair traits.

Basal roots were briefly stained with diluted Toluidine blue O (0.05%) for better visualization of root hairs. Root hair images were visualized with a light microscope (Nikon SMZ-4) and images were captured with a Nikon DS-Fi1 camera at 40x magnification and NIS-Elements F2.30 software. In addition, an image of a Hemacytometer (Hausser Scientific Horsham, PA, USA) was taken along with root hair image to be used for scale. Images were taken 2 cm above from emerging new root hairs. Image J image analysis software (http://rsbweb.nih.gov/ij/download.html) was used to measure root hair length and density. Root hair length of each genotype was measured in 5 different representative segments per replication. The root hair density was measured by counting the number of root hairs in a representative area. Root hair density was then converted to the number of root hairs per mm^2 of root surface. Genotypes were then grouped in three categories based on root hair length: short root haired (less than 0.4 mm), intermediate (0.4 - 0.6 mm) and long root haired (more than 0.6 mm).

Field experiment

The field experiment was conducted in 2010 at the Russell Larson Agricultural Research Station of The Pennsylvania State University, USA (40° 44’ N; 77° 53’ W, 366 m.a.s.l.). The genotypes were grown in a Hagerstown silt loam soil (fine, mixed, semi-active, medic Typic Hapludult). The experimental design was a randomized complete block design (RCBD) with 4 replications and each experimental unit was composed of one row of 1.6 m with eight plants. A subset of one hundred and fifty five (155) accessions from the laboratory evaluations were used for field study. Seeds of each accession were sown in one row of 1.6 m. The planting space between rows was 0.7 m and between plants in a row was 0.2 m. Each experimental unit had 8 plants. Weed and pest management and irrigation were applied as needed. The experiment was planted under low P availability, and level of P in the field was 10.5 ppm (P – Mehlich 3 extraction). Weed and pest control, and irrigation were applied as needed.

Root crowns of 3 to 4 representative plants per replication were excavated 45 days after planting (DAP), corresponding to the pod filling growth stage (R6). The root crowns had a radius of 25-30 cm around the shoot with a depth of 25-30 cm. The excavated root crowns were carefully shaken to remove excessive soil. The remaining soil was removed by soaking the root

crowns in water containing about 0.5% detergent and rinsing with water from a hose at low pressure. The following traits were measured: adventitious root length, branching and diameter; basal root length, branching and diameter, primary root length, branching and diameter; basal root angle; number of nodules; and root rot. Actual counts were taken for total number of adventitious and basal roots, and basal root whorls. Root length, angle from horizontal and diameter were measured with a ruler, protractor and caliper respectively. The root diameter was measured in the main axis and 2 cm from the base of each root class. Root branching (density) was obtained by counting the number of lateral roots in a representative segment of 2 cm in each root class, and a visual score (1 - 9) was used to rate root rot infection, where 1 corresponded to no symptoms and 9 severe symptoms. One representative score was recorded for each root trait per replication. Shoots of 3 plants were dried at 60^{o} for 2-3 days for determination of the shoot dry weight.

Statistical analysis

The data were analyzed using Minitab statistical software (Minitab Inc., State College, PA, USA), Statistix version 8 (Analytical Software, Tallahassee, FL, USA) and R program, version 2.14.0 (The R Foundation for Statistical Computing, Vienna, Australia). Analyses of variance were performed separately for laboratory and field experiments. Laboratory and field experiments were analyzed as RCBD. Correlation analyses were performed to compare traits evaluated in seedlings in the laboratory with traits evaluated in older plants grown in the field, and to determine relationships among traits. Principal component analyses using correlation matrix and the eigenvalue associated with a principal component versus the number of the component were performed to identify root phenes that introduced most of the phenotypic variation within Andean and Mesoamerican gene pools. The first three components were described based on variable loading scores, and loading plots were constructed based on the scores of the first and second components. The first two components were characterized based on variable eigenvalues, and on vector clustering within plots of components 1 and 2. To remove the effect of plant size on trait values, allometric coefficients (Log_{10} of each trait value) were used to performed correlation analyses between root traits and shoot dry weight.

Results

Genotypic variation of root traits

Considerable variation in root traits was found within and between Andean and Mesoamerican gene pools in 8 day old seedlings. Significant differences among 165 genotypes were detected in BRWN ($F = 8.22$, $p < 0.001$), number of basal roots ($F = 7.71$, $p < 0.001$) and root hair length ($F = 6.33$, $p < 0.001$) evaluated in 8 day old seedlings. BRWN varied from 1 to 4. Most genotypes had 2 whorls (data not shown). The average root hair length measured on basal roots varied from 0.19 to 0.78 mm (Figure 3-1). From a total of 165 genotypes 21 % of the genotypes had long root hairs (> 0.6 mm), 49 % had intermediate root hair length (0.4-0.6 mm), and 30 % had short root hairs (less than 0.4 mm). Among accessions with long root hairs, 86.2 % were from the Mesoamerican gene pool and only 13.8 % (corresponding to 5 genotypes out of 36) were from the Andean gene pool.

Large variation was found in root traits evaluated in the field within Andean (Figure 3-2) and within Mesoamerican (Figure 3-3) gene pools. Field measurements showed significant differences in root traits between gene pools, races and country of origin of the genotypes for most traits (Table 3-1). Variation among genotypes within gene pool, genotypes within race, and genotypes within country of origin were significant for all traits (Table 3-1).

Andean genotypes had greater means for basal root whorl number, basal root number, branching and diameter, primary root diameter, and number of nodules, while the mean of Mesoamerican genotypes were greater for adventitious root number and basal root growth angle, and these means were statistically different (Table 3-2, Appendix 3-2). Means of Andean races NG2, P1, NG1 were superior and statistically different from races from Mesoamerican gene pool for adventitious root branching, basal root whorl number, basal root number and basal root branching (Table 3-1, Appendix 3-2). Means for basal root diameter, basal root growth angle and primary root branching among races were not statistically different (Appendix 3-2).

Principal component analyses were performed separately on Andean and Mesoamerican gene pools. The first three components accounted for 51.9 % of the total variation on data set from Andean gene pool and 48.1 % of the total variation on Mesoamerican gene pool (Table 3-3 and 3-4, Figure 3-4 and 3-5). Variables with high loading scores in the first and second component mostly corresponded to root traits from similar root classes in both gene pools. Variables associated with the first component of the Andean gene pool were adventitious roots, basal roots, and primary root diameter. For the Mesoamerican gene pool, the variables associated with the first component included adventitious roots and basal roots. Traits associated with the second component were adventitious, basal, and primary roots for the Andean gene pool and adventitious and basal roots for the Mesoamerican gene pool.

Correlations among traits

High correlation between basal root number and basal root whorl number evaluated in 8 day old seedlings was found ($R^2 = 0.9$, $p < 0.0001$). Basal root number evaluated in 45 day old plants in the field was positively correlated with BRWN measured in 8 day old seedlings in the laboratory ($R^2 = 0.522$, $p < 0.001$). Pearson correlation analyses between selected root traits measured in the field varied from weak to high (Figure 3-6). Similarly to data from 8 day old seedlings, a strong and significant correlation was found between basal root number and BRWN measured in the field ($R^2 = 0.9$, P-value < 0.0001). Basal root growth angle was negatively correlated to basal root number and BRWN. Tradeoffs between adventitious root number and basal root number and between adventitious root number and BRWN were detected (Figure 3-6). In addition, a subset of 10 genotypes was selected to assess the correlation between root hair length and density measured on basal roots of 8 day old seedlings. Root hair length and density were strongly correlated ($R^2 = 0.69$, $p < 0.001$) (Figure 3-7).

Allometric analysis of shoot dry weight and selected root traits were performed separately for genotypes with growth habit Type 1, Type 2, and Type 3. Types 1 and 3 were composed of genotypes from Andean and Mesoamerican gene pools, respectively, and Type 2 was a mix of both gene pools. Type 1 growth habit corresponds to bush beans with determinate growth, Type 2 genotypes are indeterminate upright bush beans, and Type 3 are indeterminate

semi-viney prostrate beans (Beebe, 2000). Correlation between shoot dry weight and selected root traits were not significant for all root traits except adventitious root number ($R^2 = 0.11$, significant at 5%) in Type 2 growth habit (Figures 3-8, 3-9, 3-10). These results indicate that no relationship was detected between shoot dry weight and evaluated root traits in this environment.

Discussion

In this study we assessed the genetic diversity of root traits of common bean accessions from the two major gene pools, Andean and Mesoamerican. These gene pools correspond to two distinct geographic regions of common bean domestication (Gepts, 1988; Pachico, 1989; Gepts and Debouck, 1991; Singh et al., 1991a, Singh et al., 1991b; Singh et al., 1991c; Noradi et al., 1992; Broughton et al., 2003; Zizumbo-Villarreal et al., 2005). We found considerable variation in root traits between and within accessions from Andean and Mesoamerican gene pools. The high diversity in root traits found in this study is probably associated with the diverse genetic background that exists in *Phaseolus vulgaris*. The genotypes evaluated in the present study were from 15 countries and they belong to 8 different bean races: 3 races from Andean gene pool and 5 from Mesoamerican gene pool (Appendix 3-1). Differences in plant morphology, growth habit, seed size and color were evident among the genotypes and as expected, we found substantial differences in the root architectural phenotype as well. High genetic diversity in plant and seed morphology (Singh et al., 1991a), chloroplast DNA (Chacón et al., 2005), phaseolin seed protein (Gepts, 1988) in common bean has been reported. The diverse genetic variation in root traits we found may be associated with plant adaptation to different agro-ecological conditions in the Mesoamerican and Andean regions. Our results indicate that both Andean and Mesoamerican genotypes evolved adaptations to low P availability as demonstrated by the greater number of whorls and basal roots, proliferation of lateral roots and shallower basal roots found in the Andean races, and the large number of adventitious roots, lateral branches and long and dense root hairs present in the Mesoamerican races. The proliferation of adventitious roots in beans have been reported to be associated with adaptation to low P soils (Miller et al., 2003, Ochoa et al., 2006, Walk et al. 2006).

The mean of basal root growth angle of the Andean genotypes was relatively lower than the mean of the Mesoamerican genotypes, indicating that genotypes from the Andean gene pool have relatively shallower basal roots than genotypes from the Mesoamerican gene pool. Andean genotypes had more whorls and basal roots. A greater number of basal roots have been found to be associated with shallower basal roots (Basu, 2007), a trait associated with P stress adaptation. Genotypes with shallower basal roots were reported to take up more P under P stress (Rubio et al, 2003, Ho et al., 2005, Liao et al. 2001). Overall, our data suggest that genotypes from Andean gene pool have root traits that are suitable for regions with low P availability, therefore, genotypes with superior root traits could be targeted in breeding programs for development of varieties tolerant to low P stress. On the other hand, it appears that races and most accessions from the Mesoamerican regions developed adaptations to drought stress. Our accessions from the Mesoamerican origin had 2 whorls, deeper basal root growth angles than those from the Andean gene pool, and more lateral branches on primary roots. Steeper basal root angle often results in the development of deep roots that are associated with drought tolerance because these roots may acquire water from deeper soil profiles. In fact, most of the accessions from Mesoamerican gene pool were from races M1 (Mesoamerica) reported to be tolerant to drought stress and low soil fertility and D (Durango) that are common in semi-arid regions of Central America and reported to be tolerant to drought (Singh et al., 1991a). Some Andean accessions also had lateral roots on primary and basal roots, which may confer adaptation to drought stress.

Differences in root traits among accessions within gene pools may be due to differences in agro-ecological adaptation in addition to genetic differences. In fact, the criteria used to distinguish races in common bean include agro-ecological adaptation, differences in plant and see morphology such as growth habit (Singh et al. 1991a). Our data suggest that sources of tolerance to low P and drought stress could be found in both Andean and Mesoamerican gene pools. This information is important for breeding programs for development of bean varieties for specific regions. For instance, genotypes with many long and well branched adventitious roots, shallow basal roots, several basal root whorls and several lateral roots on basal or adventitious roots and longer and denser root hairs will have advantage in exploiting the topsoil where P is concentrated. In contrast, genotypes with long and well branched primary roots and steeper basal root angles will be able to acquire water in deep soil horizons. Combinations of traits such as long and dense root hairs with deep basal roots, or many adventitious roots with long primary root may potentially result in increased crop performance and yield in regions where multiple stresses such

as low P availability and drought occur. Since phenotypic diversity was found within races of Andean and Mesoamerican gene pools, both crosses within and between gene pools will be useful to improve tolerance to edaphic stresses in beans.

The method used to excavate root crowns in the present study did not provide measurements of the entire length of basal, primary and adventitious roots because many of these roots were severed during excavation. However, the data gives a good estimation of the distribution of these roots within the root crown area as illustrated by the frequency distributions (Figure 3-2 and 3-3) and the significant differences among genotypes (Table 3.1). Additional information to confirm the positive correlation between steeper root angles and deeper basal roots and root lengths requires evaluation of root segments present in different soil depths by taking soil cores. Changes in growth angle of basal roots in response to low P availability have been reported. Bonser et al. (1996) reported a decrease in root growth angle in some common bean genotypes under P deficiency. Liao et al. (2001) also observed changes in basal root growth angle in response to phosphorus availability.

Principal component analyses showed that 51.9 % of the total variation on Andean gene pool was explained by components 1, 2 and 3. In the Mesoamerican gene pool components 1, 2 and 3 accounted for 48.1 % of the total variation. Variables with high loading scores in the first and second components were similar in both Andean and Mesoamerican gene pools. Based on these results we could conclude that the first component corresponds to traits associated with adventitious and basal root classes since the variables associated with this component were mostly traits from adventitious and basal root classes in both gene pools. Variables associated with the second component were interpreted as traits from adventitious, basal and primary root classes in the Andean gene pool, and adventitious root class in the Mesoamerican gene pool. These results indicate that most of the variation in the data set from each gene pool was mostly introduced by adventitious and basal root classes and a smaller proportion by the primary root.

Strong correlation was detected between basal root number and basal root whorl number both in 8 day old seedlings and in 45 day old plants, indicating that BRWN measured in seedlings can predict the total number of basal roots in later growth stages; therefore, screening for BRWN and basal root number in 3 day old seedlings would save time and resources. Our results detected positive correlation between root hair length and root hair density, traits that confer P acquisition

efficiency in crops, in accord with previous reports (eg Gahoonia and Nielsen, 1997, Ma et al., 2001, Gahoonia et al., 2004; Vieira et al., 2007). We found tradeoffs between adventitious root number and basal root number and between adventitious root number and BRWN, as illustrated by the negative correlation between these root traits (Figure 3-6). Walk et al. (2006) reported that the metabolic investment in adventitious roots may retard the development of basal roots. This information is relevant for breeding crops with traits adapted to low P availability and drought.

Weak to no correlations were detected between allometric coefficients of selected root traits and shoot dry weight (Figure 3-8, 3-9 and 3-10). Our data showed that shoot dry weight at 45 days after planting was independent from the root traits. We expected an increase in shoot dry weight in genotypes with root traits that confer P efficiency under P stress. One possible explanation for the weak correlation between shoot dry weight and root traits is the genetic differences in plant size and vigor that exists in beans even within genotypes of the same growth habit group. Another possible explanation for the weak correlation is the level of P stress in the field. The level of P in the field ranged from 9 to 12 ppm, and it was probably high enough to not cause P stress in plants. In fact, most plants did not show severe symptoms of P deficiency at the time of evaluation, even the check genotypes DOR 364 that was planted in every 5 rows of the experiment did not show severe symptoms of P deficiency. Our results indicate that plants of any size with indeterminate or determinate growth habit may have for instance more or less BRWN, therefore, root traits conferring adaptation to low P stress can be found in genotypes of any growth habit and size.

Conclusion

From this study, we can conclude that there is substantial phenotypic variation in root traits between bean accessions within and between the Andean and Mesoamerican gene pools. Overall, genotypes with more adventitious roots and longer root hairs were found in races from the Mesoamerican gene pool, and genotypes with more basal root whorls, more basal roots and relatively shallower basal root growth angle were found in races from the Andean gene pool. These root traits are important for P acquisition under low P conditions because they enhance topsoil foraging. Genotypes with steep basal root growth angles that are often associated with

deeper basal roots were found in races from Mesoamerican gene pool, while genotypes with extensive primary and basal roots were found in races from the Andean gene pools. These traits are associated with drought tolerance. Information presented in this study indicates that sources of useful root traits conferring tolerance to low P and drought stresses can be found in accessions from both Andean and Mesoamerican genotypes. Information on genotypic diversity of root traits and sources of useful root traits is important in breeding programs for development of genotypes adapted to specific stress. Breeding for multiple root traits could enhance acquisition of multiple soil resources. Field phenotyping could be an appropriate tool for bean breeding for low P and drought tolerance in developing countries.

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Table 3-1. Analyses of variance of 16 traits evaluated in the field, showing F values and levels of significance. Adventitious root number (ARN), adventitious root length (ARL), adventitious root branching (ARB), adventitious root diameter (ARD), basal root whorl number (BRWN), basal root number (BRN), basal root length (BRL), basal root branching (BRB), basal root diameter (BRD), basal root growth angle, primary root length (PRL), primary root branching (PRB), primary root diameter (PRD), and shoot dry weight in grams (SDW). Lengths and diameters are in cm and mm, respectively. Branching corresponds to number of lateral roots in a root segment of 2 cm. ARN, BRWN and BRN are counts per plant. G. = Genotype. Gene pool: Andean and Mesoamerican. Origin of the accessions: Colombia, Guatemala, Brazil, Costa Rica, Mexico, Peru, Haiti, Jamaica, Nicaragua, Ecuador, El Salvador, Chile, Cuba, Dominican Republic, United States of America. Races: NG1-Nueva Guarda, group 1, NG2-Nueva Guarda, group 2, P1-Peru, group 1, G-Guatemala, D1-Durango, group 1, D2-Durango, group 2, M1-Mesoamericana, group 1, M2-Mesoamericana, group 2. Levels of significance: *** - significant at 1%; ** - significant at 5%; * - significant at 10%, ns – not significant.

	ARN	ARL	ARB	ARD	BRWN	BRN	BRL	BRB	BRD	BRGA	PRL	PRB	PRD	Nod	SDW	RR
Source of variation							F values									
Gene pool	37.2 ***	0.4 ns	6.53 **	24.9 ***	67.5 ***	44.9 ***	2.71 ns	7.04 ***	12.2 ***	33.9 ***	2.57 ns	7.9 **	13.1 ***	13.8 ***	0.12 ns	36.7 ***
G. within gene pool	5.52 ***	2.47 ***	2.3 ***	2.21 ***	3.07 ***	2.92 ***	1.44 ***	1.66 ***	1.68 ***	2.34 ***	1.82 ***	1.7 ***	2.37 ***	2.21 ***	1.31 ***	1.31 *
Origin	1.02 ns	1.54 *	3.13 ***	2.03 **	2.51 ***	2.21 ***	1.89 **	1.39 ns	1.47 ns	1.65 *	1.23 ns	3.71 ***	0.56 ns	3.33 ***	1.0 ns	1.27 ns
G. within origin	6.8 ***	2.35 ***	1.99 ***	2.34 ***	3.86 ***	3.39 ***	1.35 **	1.66 ***	1.73 ***	2.68 ***	1.8 ***	1.42 ***	2.58 ***	1.96 ***	1.62 ***	1.57 ***
Race	4.22 ***	1.14 ns	2.76 ***	4.16 ***	12.5 ***	8.62 ***	1.02 ns	3.09 ***	2.00 **	3.33 ***	0.49 ns	2.30 **	1.91 *	4.29 ***	1.97 *	5.95 ***
Genotype within race	5.95 ***	2.45 ***	2.2 ***	2.23 ***	2.88 ***	2.79 ***	1.46 ***	1.57 ***	1.72 ***	2.56 ***	1.89 ***	1.67 ***	2.38 ***	2.07 ***	1.55 ***	1.31 **

Table 3-2 Means and standard errors (SE) of 16 traits evaluated in 155 Andean (A) and Mesoamerica (M) genotypes. Means and SE of 8 races are presented. Adventitious root number (ARN), length (ARL), branching (ARB) and diameter (ARD); Basal root whorl number (BRWN); Basal root number (BRN), length (BRL), branching (BRB) and diameter (BRD); Basal root growth angle (BRGA); Primary root length (PRL), branching (PRB) and diameter (PRD); number of nodules per plant (Nod); 1 to 9 root rot infection score (RR), and shoot dry weight in grams (SDW). Branching correspond to number of lateral roots in 2 cm root segment. ARN, BRWN and BRN are counts per plant. Root length and diameter are in cm and mm, respectively. Races: NG1-Nueva Guarda, group 1, NG2-Nueva Guarda, group 2, P1-Peru, group 1, G-Guatemala, D1-Durango, group 1, D2-Durango, group 2, M1-Mesoamericana, group 1, M2-Mesoamericana, group 2.

	ARN	ARL	ARB	ARD	BRWN	BRN	BRL	BRB	BRD	BRGA	PRL	PRB	PRD	Nod	SDW	RR
Mean and SE of gene pools																
A	15.82	13.97	6.28	0.70	2.46	8.82	21.86	6.92	2.24	37.98	17.14	7.54	2.47	32.70	113.08	1.62
SE	0.45	0.37	0.14	0.03	0.03	0.12	0.37	0.15	0.05	1.16	0.35	0.15	0.05	1.98	0.07	0.07
M	23.28	13.55	5.64	0.50	2.00	7.48	22.68	6.39	2.02	49.51	16.40	6.94	2.74	22.95	111.70	1.21
SE	0.51	0.31	0.12	0.02	0.02	0.08	0.26	0.10	0.03	0.91	0.21	0.10	0.05	1.09	1.85	0.03
Mean and SE of races																
D1	23.98	14.04	5.48	0.43	1.87	6.92	21.04	6.33	1.91	52.79	16.42	6.94	2.44	23.08	106.21	1.23
SE	1.62	0.79	0.31	0.04	0.06	0.22	0.72	0.28	0.08	2.37	0.47	0.29	0.11	2.96	4.64	0.08
D2	22.27	14.00	5.88	0.53	2.02	7.55	23.50	5.77	1.99	46.17	16.67	6.77	2.80	20.80	116.15	1.25
SE	1.04	0.69	0.27	0.05	0.06	0.22	0.68	0.23	0.08	2.51	0.55	0.26	0.12	2.62	5.12	0.07
G	21.75	13.53	5.45	0.49	2.05	7.50	22.55	6.78	2.11	47.63	16.40	7.40	2.71	22.75	109.43	1.15
SE	1.57	0.92	0.30	0.06	0.05	0.20	0.84	0.36	0.10	3.32	0.74	0.38	0.15	2.67	5.10	0.08
M1	23.94	13.07	5.26	0.50	1.83	7.02	22.74	6.36	2.00	50.91	16.42	6.60	2.81	20.53	108.13	1.10
SE	1.16	0.75	0.29	0.04	0.04	0.16	0.56	0.21	0.07	1.96	0.48	0.20	0.11	2.17	3.52	0.05
M2	22.09	12.94	5.32	0.47	2.07	7.73	22.85	6.47	2.07	48.41	16.00	7.02	2.87	22.36	119.93	1.22
SE	0.84	0.65	0.22	0.03	0.03	0.13	0.55	0.17	0.06	1.71	0.37	0.20	0.09	2.14	4.16	0.05
NG1	13.98	12.56	6.14	0.62	2.53	8.96	21.90	6.36	2.29	37.60	17.26	6.99	2.52	26.14	118.78	1.75
SE	0.63	0.50	0.21	0.03	0.05	0.19	0.52	0.20	0.07	1.74	0.58	0.21	0.07	2.73	4.39	0.12
NG2	18.90	14.94	6.58	0.70	2.31	8.34	22.44	7.18	2.20	42.33	16.94	7.72	2.48	31.32	107.85	1.48
SE	0.89	0.49	0.23	0.04	0.05	0.17	0.47	0.21	0.06	1.70	0.43	0.20	0.07	2.33	2.89	0.08
P1	22.30	14.79	6.41	0.73	2.45	8.96	21.68	7.14	2.06	42.23	17.02	7.73	2.54	44.48	105.78	1.38
SE	1.25	0.71	0.26	0.06	0.07	0.24	0.81	0.34	0.09	2.18	0.65	0.29	0.11	4.68	3.25	0.10

Table 3-3. Principal component analysis for traits evaluated on accessions from the Andean gene pool. Loading scores and variance proportion are presented for traits measured on root crown. adventitious root number (ARN), adventitious root length (ARL), adventitious root branching (ARB), adventitious root diameter (ARD), basal root whorl number (BRWN), basal root number (BRN), basal root length (BRL), basal root branching (BRB), basal root diameter (BRD), basal root growth angle (Angle), primary root length (PRL), primary root branching (PRB), primary root diameter (PRD) and shoot dry weight (SDW).

Andean	Component		
Trait	PC1	PC2	PC3
ARN	-0.020	0.333	0.201
ARL	0.240	0.190	0.289
ARB	0.280	0.306	0.269
ARD	0.305	0.122	0.276
BRWN	0.251	-0.454	0.244
BRN	0.234	-0.458	0.235
BRL	0.353	0.098	-0.153
BRB	-0.127	0.289	0.291
BRD	0.466	0.018	-0.164
BRGA	-0.248	0.136	-0.150
PRL	0.265	0.272	-0.418
PRB	0.182	0.271	0.251
PRD	0.290	0.068	-0.264
SDW	0.215	-0.255	-0.049
Proportion of variation	0.227	0.172	0.121
Cumulative proportion	0.227	0.399	0.519

Table 3-4. Principal component analysis for traits evaluated on Mesoamerican gene pool. Loading scores and variance proportion are presented for traits measured on root crown. Adventitious root number (ARN); Adventitious root length (ARL); Adventitious root branching (ARB); Adventitious root diameter (ARD); Basal root whorl number (BRWN); Basal root number (BRN); Basal root length (BRL); Basal root branching (BRB); Basal root diameter (BRD); Basal root growth angle (BRGA); Primary root length (PRL); Primary root branching (PRB); Primary root diameter (PRD); and shoot dry weight (SDW).

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Mesoamerican	Component		
Trait	PC1	PC2	PC3
ARN	0.267	0.302	0.020
ARL	0.399	-0.128	0.103
ARB	0.395	0.046	0.232
ARD	0.342	-0.196	0.273
BRWN	0.089	-0.587	0.057
BRN	0.073	-0.599	0.087
BRL	-0.129	0.122	0.397
BRB	0.105	0.186	0.479
BRD	-0.362	-0.086	0.306
BRGA	0.330	0.260	0.133
PRL	-0.194	0.073	0.347
PRB	0.087	-0.073	0.327
PRD	-0.409	-0.022	0.313
SDW	-0.054	-0.030	0.159
Proportion of variation	0.198	0.165	0.118
Cumulative proportion	0.198	0.364	0.481

Table 3-5. Summary of descriptive statistics of traits evaluated in 155 genotypes from Andean (A) and Mesoamerican (M) gene pools. Traits were adventitious root number (ARN), length (ARL), branching (ARB) and diameter (ARD); Basal root whorl number (BRWN); Basal root number (BRN), length (BRL), branching (BRB), diameter (BRD) and angle (BRGA); Primary root length (PRL), branching (PRB) and diameter (PRD); number of nodules (N. nodules), and root rot infection (Rrot) (1-9 score) and shoot dry weight (SDW). Branching correspond to number of lateral roots in 2cm root segment. ARN, BRWN and BRN are counts per plant. The 1-9 scores a described in Table 1.

Variable	Gene pool	Mean	SE mean	STDev	Minimum	Median	Maximum
ARN	A	15.82	0.45	6.69	2.0	16	46
	M	23.28	0.51	10.19	5.0	21	62
ARL (cm)	A	13.97	0.37	5.45	3.0	14	30
	M	13.55	0.31	6.11	1.0	14	35
ARB	A	6.28	0.14	2.11	1.0	6.0	14
	M	5.64	0.12	2.42	1.0	5.0	16
ARD (mm)	A	0.70	0.03	0.38	0.1	0.6	2.2
	M	0.49	0.02	0.37	0.1	0.4	2.0
BRWN	A	2.46	0.03	0.52	1.0	2.0	3.0
	M	2.00	0.02	0.45	1.0	2.0	3.0
BRN	A	8.82	0.12	1.82	4.0	8.0	12
	M	7.48	0.08	1.63	4.0	8.0	12
BRL (cm)	A	21.86	0.37	5.45	3.0	22	39
	M	22.68	o.26	5.29	2.0	22.5	37
BRB	A	6.92	0.15	2.23	3.0	7.0	18
	M	6.39	0.10	2.05	2.0	6.0	14
BRD (mm)	A	2.24	0.05	0.67	0.7	2.2	4.1
	M	2.02	0.03	0.63	0.2	2.0	4.0
BRGA (^{o})	A	37.98	1.16	17.22	10	40	80
	M	49.51	0.91	18.17	10	50	80
PRL (cm)	A	17.14	0.35	5.14	6.0	16	35
	M	16.39	0.21	4.25	4.0	16	30
PRB	A	7.54	0.15	2.27	3.0	7.0	18
	M	6.94	0.10	2.05	3.0	7.0	13
PRD (mm)	A	2.47	0.05	0.72	0.8	2.4	4.2
	M	2.74	0.05	0.92	0.5	2.7	6.6
N. nodules	A	32.70	1.98	29.4	0.0	25.0	145
	M	22.95	1.09	21.7	0.0	15.0	130
Rrot(score)	A	1.62	0.07	1.01	1.0	1.0	6.0
	M	1.20	0.03	0.59	1.0	1.0	5.0
SDW (g)	A	113.87	2.57	31.83	30.4	111.7	213.9
	M	111.70	1.85	32.03	46.8	111.9	210.0

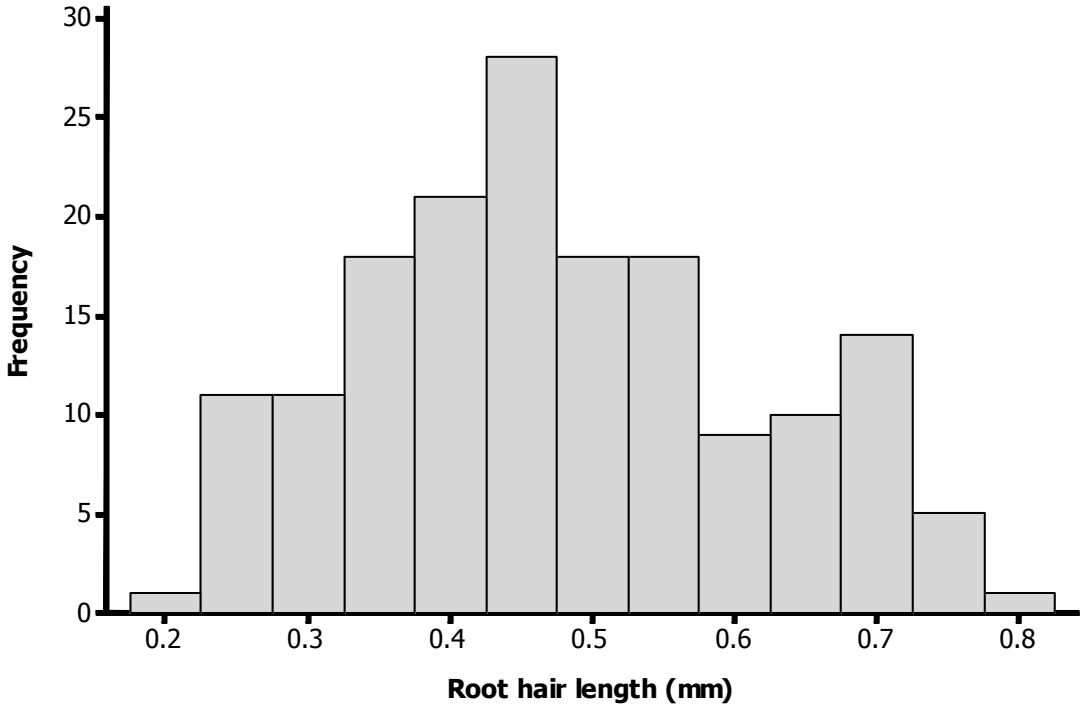

Figure 3-1. Phenotypic variation of root hair length in 165 accessions from the Andean and Mesoamerican gene pools measured in 8 day old bean seedlings. The data are average of 4 replications.

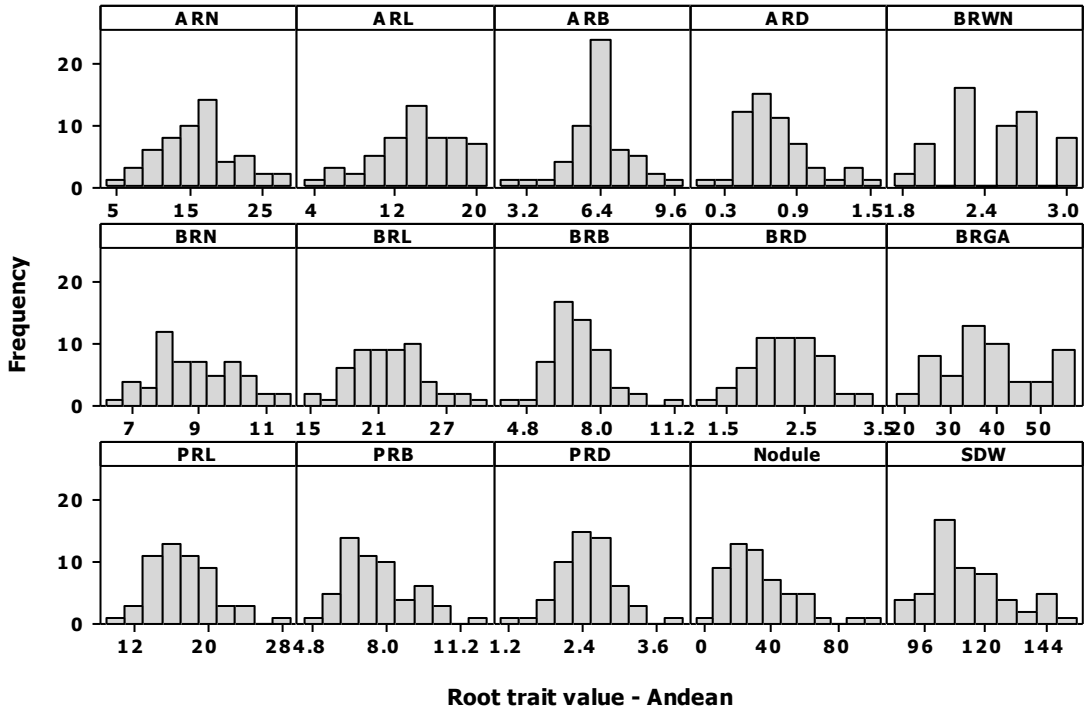

Figure 3-2. Phenotypic variation of root traits in accessions from the Andean gene pool evaluated in the field in Rock Springs, 2010. Adventitious root number (ARN); Adventitious root length (ARL); Adventitious root branching (ARB); Adventitious root diameter (ARD); Basal root whorl number (BRWN); Basal root number (BRN); Basal root length (BRL); Basal root branching (BRB); Basal root diameter (BRD); Basal root growth angle (BRGA); Primary root length (PRL); Primary root branching (PRB); Primary root diameter (PRD); number of nodules per plant, and shoot dry weight in grams (SDW). Branching correspond to number of lateral roots in 2 cm root segment. ARN, BRWN and BRN are counts per plant. Root length and diameter are in cm and mm, respectively. The data are average of 4 replications.

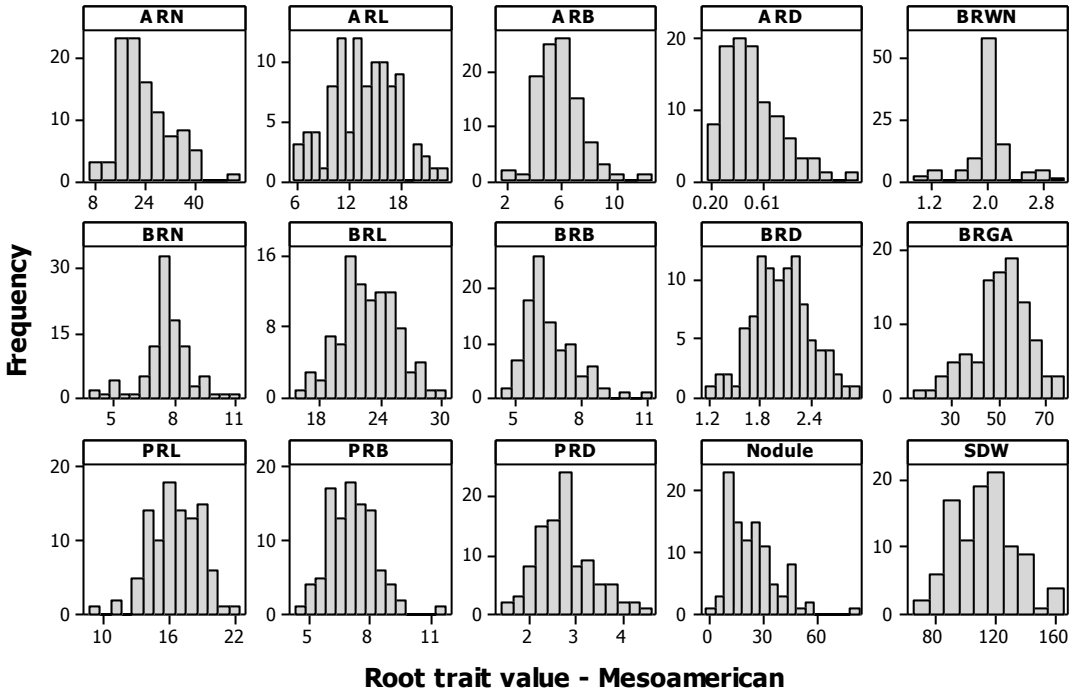

Figure 3-3. Phenotypic variation of root traits in accessions from the Mesoamerican gene pool. Adventitious root number (ARN); Adventitious root length (ARL); Adventitious root branching (ARB); Adventitious root diameter (ARD); Basal root whorl number (BRWN); Basal root number (BRN); Basal root length (BRL); Basal root branching (BRB); Basal root diameter (BRD); Basal root growth angle (BRGA); Primary root length (PRL); Primary root branching (PRB); Primary root diameter (PRD); number of nodules per plant, and shoot dry weight in grams (SDW). Branching correspond to number of lateral roots in 2cm root segment. ARN, BRWN and BRN are counts per plant. Root length and diameter are in cm and mm, respectively. The data are average of 4 replications.

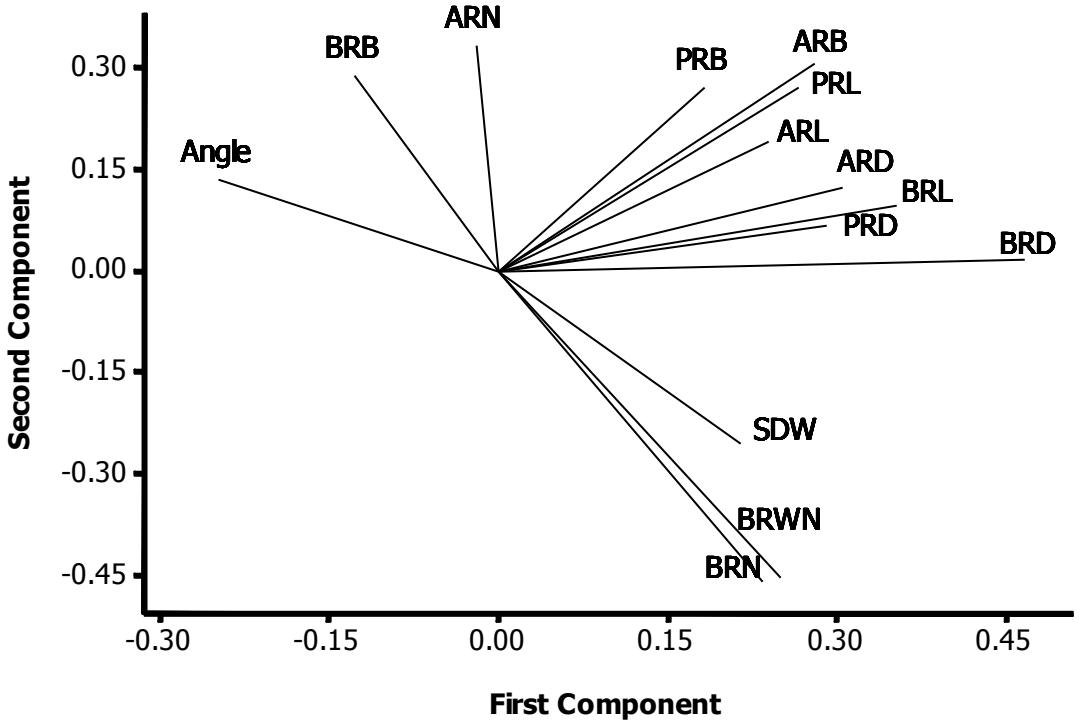

Figure 3-4. Loading plot of root phenes from the Andean accessions. Based on the highest loading scores, the first component is mainly associated with adventitious and basal root classes and the second component is associated with adventitious, basal and primary root classes.

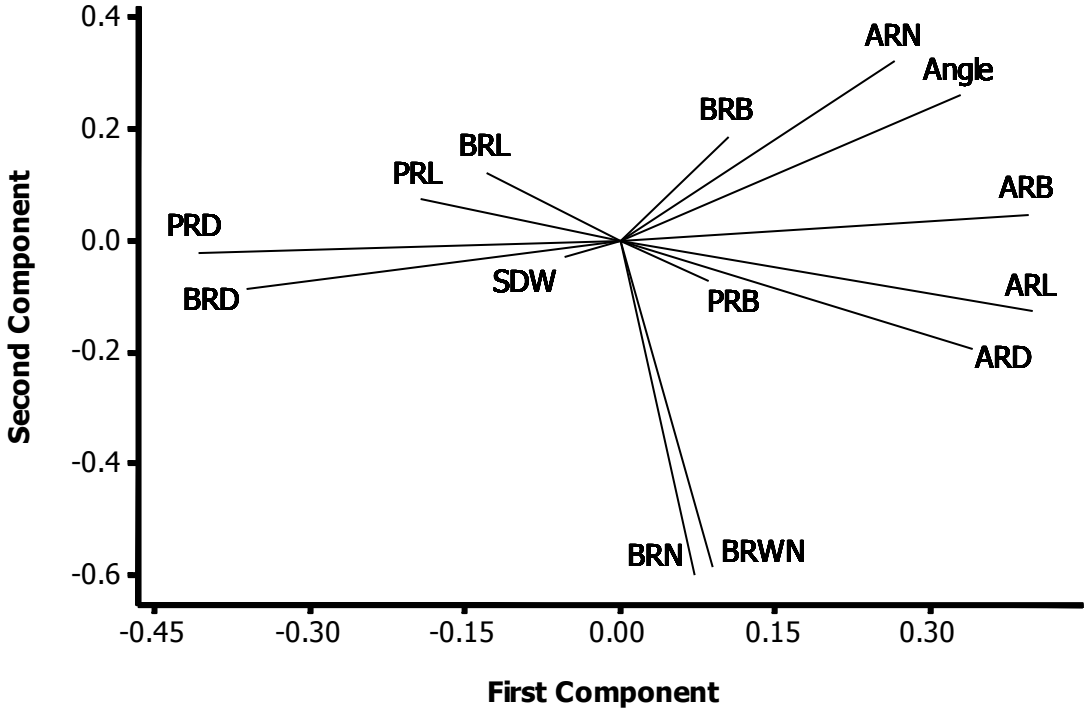

Figure 3-5. Loading plot of root phenes from the Mesoamerican accessions. Based on the highest loading scores, the first component is mainly associated with adventitious and basal root classes and the second component is associated with adventitious and basal root classes

	ARN	ARL	ARB	ARD	BRWN	BRN	BRL	BRB	BRD	BRGA	PRL	PRB	PRD	Nodule
ARL	0.088													
	0.279													
ARB	0.178	0.419												
	0.027	0.000												
ARD	-0.168	0.468	0.390											
	0.037	0.000	0.000											
BRWN	-0.386	0.119	0.124	0.356										
	0.000	0.141	0.125	0.000										
BRN	-0.367	0.129	0.076	0.321	0.942									
	0.000	0.110	0.347	0.000	0.000									
BRL	0.051	0.055	-0.036	0.095	-0.086	-0.065								
	0.530	0.499	0.660	0.240	0.290	0.420								
BRB	0.120	-0.092	0.249	0.132	0.009	-0.031	0.058							
	0.137	0.253	0.002	0.102	0.909	0.698	0.476							
BRD	-0.248	0.003	0.055	0.243	0.230	0.213	0.337	-0.028						
	0.002	0.974	0.494	0.002	0.004	0.008	0.000	0.728						
BRGA	0.404	0.127	0.059	-0.085	-0.383	-0.391	0.065	0.017	-0.398					
	0.000	0.115	0.463	0.293	0.000	0.000	0.423	0.833	0.000					
PRL	-0.104	0.057	0.030	0.056	0.001	-0.013	0.277	0.044	0.323	0.012				
	0.196	0.478	0.716	0.487	0.992	0.872	0.000	0.583	0.000	0.886				
PRB	-0.086	0.091	0.298	0.234	0.139	0.164	-0.021	0.293	0.069	-0.133	0.096			
	0.289	0.258	0.000	0.003	0.084	0.042	0.794	0.000	0.393	0.100	0.236			
PRD	-0.089	-0.262	-0.144	-0.235	-0.153	-0.127	0.266	-0.098	0.414	-0.145	0.303	0.051		
	0.273	0.001	0.073	0.003	0.058	0.115	0.001	0.227	0.000	0.071	0.000	0.532		
Nodule	0.060	0.262	0.413	0.414	0.175	0.138	0.028	0.319	0.098	-0.076	0.045	0.165	-0.268	
	0.462	0.001	0.000	0.000	0.029	0.087	0.731	0.000	0.226	0.349	0.578	0.041	0.001	
Rt rot	-0.298	0.149	0.106	0.354	0.413	0.375	0.099	0.012	0.344	-0.245	0.066	0.015	-0.095	0.048
	0.000	0.064	0.189	0.000	0.000	0.000	0.219	0.879	0.000	0.002	0.417	0.855	0.239	0.553

| | ARN | ARL | ARB | ARD | BRWN | BRN | BRL | BRB | BRD | BRGA | PRL | PRB | PRD | Nodule |

Figure 3-6. Pearson correlation coefficients and P-values for the following selected root phenes: Adventitious root number (ARN), Adventitious root length (ARL), Adventitious root branching (ARB), Adventitious root diameter (ARD), Basal root whorl number (BRWN), Basal root number (BRN), Basal root length (BRL), Basal root diameter (BRD), Basal root growth angle (BRGA), Primary root length (PRL), Primary root branching (PRB), Primary root diameter (PRD), number of nodules per plant (Nodule), and root rot infection (Rt rot). The data are average of 4 replications and 155 genotypes from both Andean and Mesoamerican gene pools. Strong correlations and tradeoff between traits are highlighted in red.

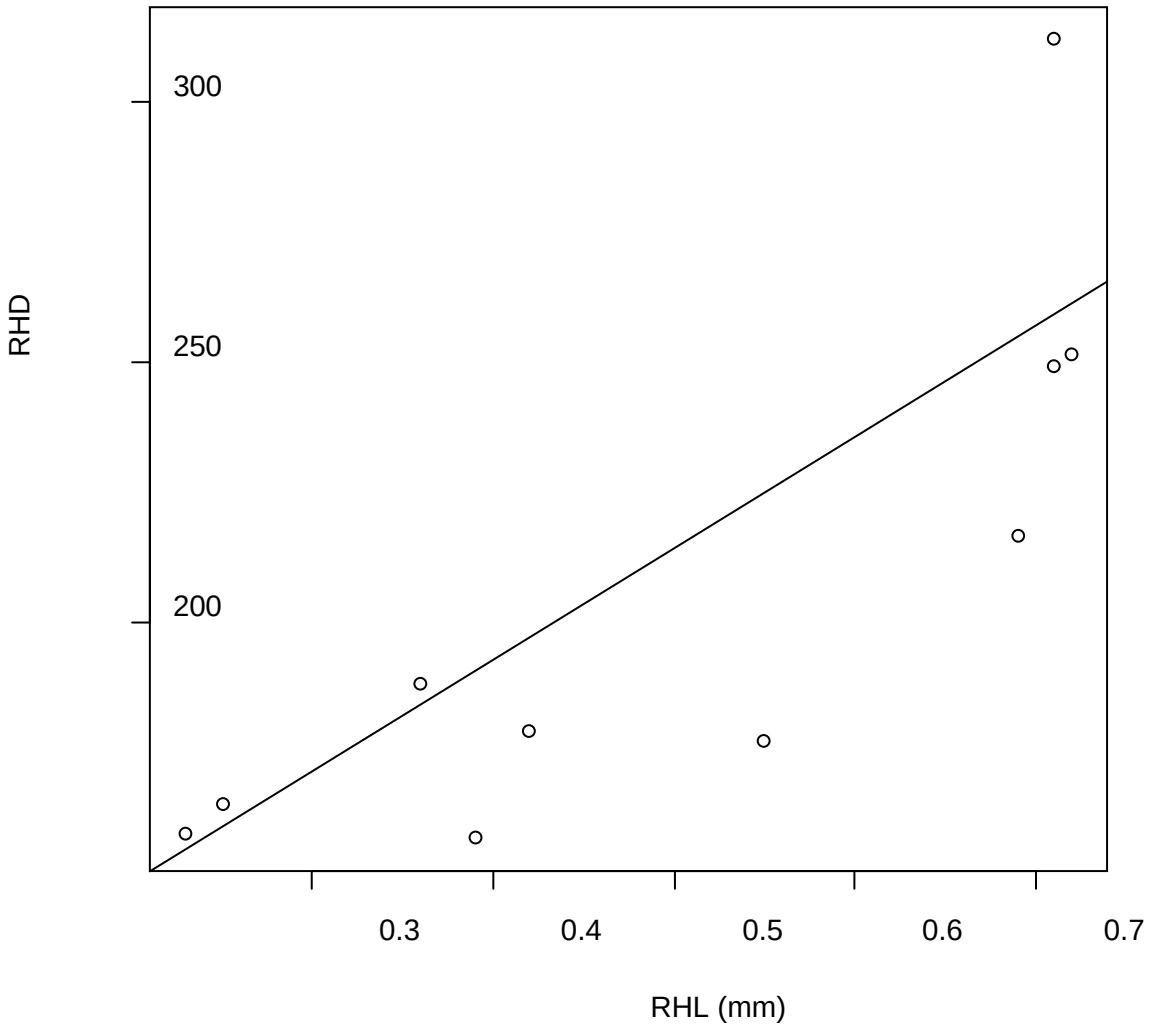

Figure 3-7. Scatterplot showing positive correlation between root hair length and root length density on selected accessions measured in 8 day old common bean seedlings. RHD = Root hair density (Number of root hair in a square millimeter). RHL = Root hair length in mm. Each point represents an average of 4 replications. $R^2 = 0.69$, $P < 0.001$. The pints are average of 4 replications.

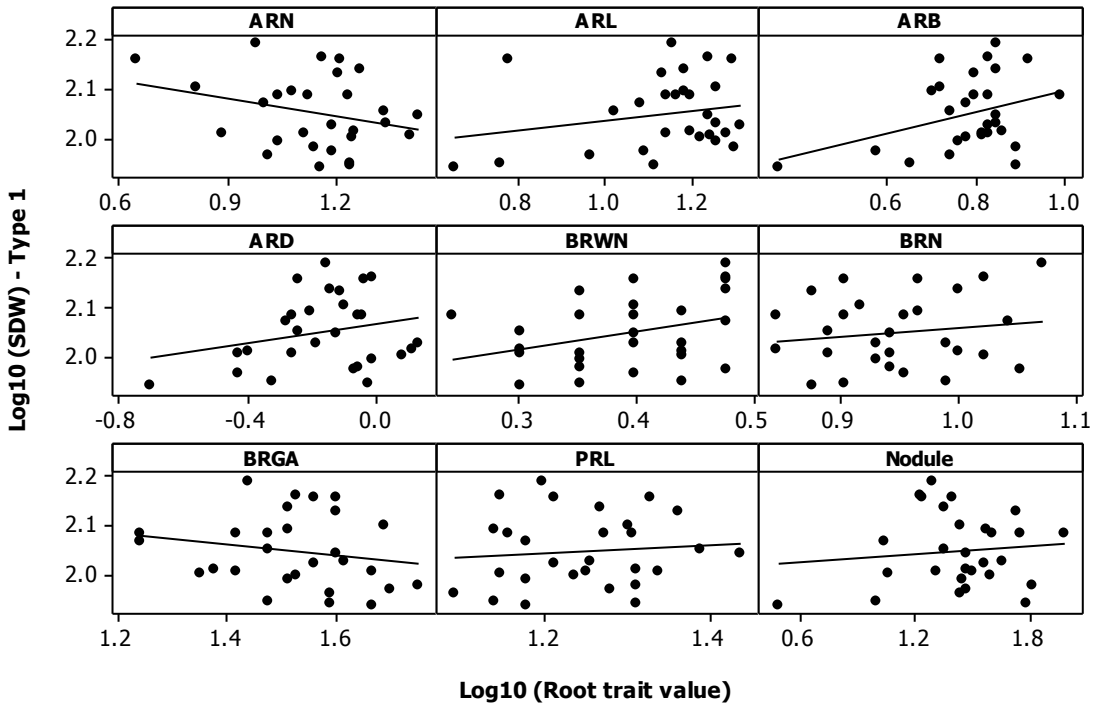

Figure 3-8. Scatterplots showing relationship between allometric coefficients of selected root phenes with allometric coefficient of shoot dry weight of accessions from Andean gene pool with Type 1 growth habit (Determinate bush). Adventitious root number (ARN) (R^2 = 0.08), Adventitious root length (ARL) (R^2 = 0.05), Adventitious root branching (ARB) (R^2 = 0.13*), Adventitious root diameter (ADR) (R^2 = 0.06), Basal root whorl number (BRWN)(R^2 = 0.11*), Basal root number (BRN) (R^2 = 0.03), Basal root growth angle (BRGA)(R^2 = 0.04), Primary root length (PRL)(R^2 = 0.01), and number of nodules per plant (R^2 = 0.013). Regression analyses were statistically significant for ARB and BRWN at 10% level of significance (*).

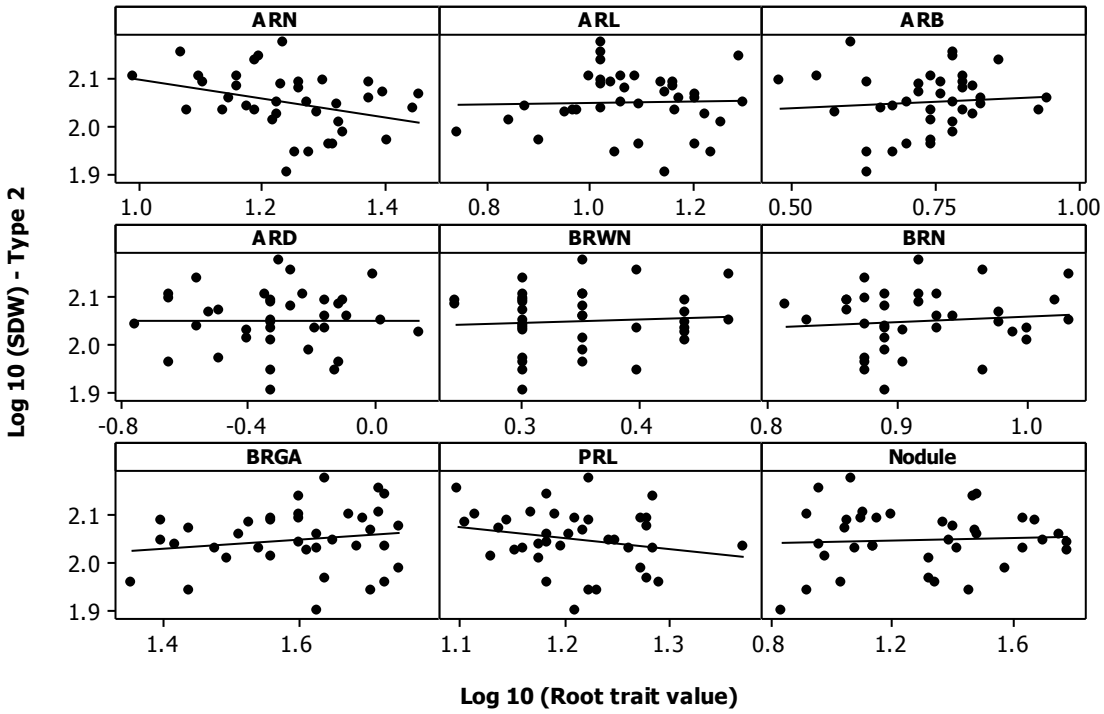

Figure 3-9. Correlations between allometric coefficients of selected root phenes with allometric coefficient of shoot dry weight of accessions from Andean and Mesoamerican gene pools with Type 2 growth habit (indeterminate upright bush). Adventitious root number (ARN) (R^2 = 0.11**), Adventitious root length (ARL) (R^2 = 0.001), Adventitious root branching (ARB) (R^2 = 0.008), Adventitious root diameter (ADR) (R^2 = 0.0), Basal root whorl number (BRWN)(R^2 = 0.004), Basal root number (BRN) (R^2 = 0.011), Basal root growth angle (BRGA)(R^2 = 0.25), Primary root length (PRL)(R^2 = 0.46), and number of nodules per plant (R^2 = 0.004). Regression analyses were only statistically significant for ARN at 5% level of significance (**). Each point represents an average of 4 replications.

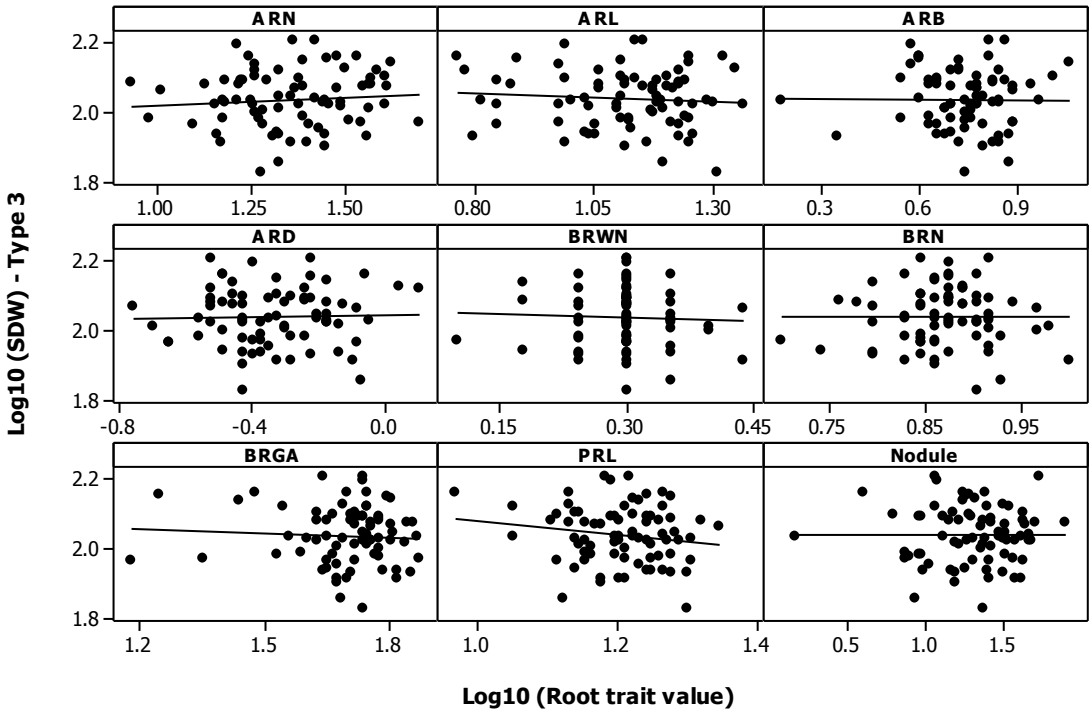

Figure 3-10. Scatterplots illustrating relationship between allometric coefficients of selected root traits and allometric coefficient of shoot dry weight of accessions from Mesoamerican gene pool with Type 3 growth habit (indeterminate semi-viney prostrate). Adventitious root number (ARN) (R^2 = 0.008), Adventitious root length (ARL) (R^2 = 0.007), Adventitious root branching (ARB) (R^2 = 0.0), Adventitious root diameter (ADR) (R^2 = 0.001), Basal root whorl number (BRWN) (R^2 = 0.001), Basal root number (BRN) (R^2 = 0.0), Basal root growth angle (BRGA)(R^2 = 0.004), Primary root length (PRL)(R^2 = 0.25), and number of nodules per plant (R^2 = 0.0). Regression analyses were not statistically significant for all traits. Each point represents an average of 4 replications.

Chapter 4

Heritability of root hair traits in common bean (*Phaseolus vulgaris* L.)

Abstract

Low P availability is a primary constraint to common bean production in many developing countries. Genotypic variation in the length and density of root hairs is associated with P acquisition and is an attractive target for bean breeding. Information on the heritability of root hair traits is needed for development of better strategies for genetic improvement of bean varieties adapted to regions with low P soils. The objectives of this study were to assess genetic variation for root hair traits in two bean populations, and to estimate the heritability of root hair length from basal roots. Artificial crosses were performed and generations of selfing F2, F3 and F4 were developed in the field in Chokwe and Sussundenga, Mozambique. SEA 5 x SXB 418 population was composed of 86 F3 individuals and corresponding 86 F4 progeny lines; and VAX 1 x SXB 418 population was composed of 72 individuals on F3 and corresponding 72 F4 progeny lines. Large variation in root hair length and density was found among progenies in F3 and F4 generations of the SEA 5 x SXB 418 and VAX 1 x SXB 418 populations. Strong positive correlation was also observed between root hair length and density in these two populations. Narrow sense heritability (h^2) of root hair length was estimated using parent – offspring regression coefficients (b) of F4 progeny family means on F3 parental values of SEA 5 x SXB 418 and VAX 1 x SXB 418 populations. We found moderately high heritability of root hair length from basal roots in population SEA 5 x SXB 418 (h^2 = 0.69) and population VAX 1 x SXB 418 (h^2 = 0.71). The high heritability suggests that considerable progress may be expected from selection for longer root hairs in segregating beam populations. Breeding for longer and denser root hairs could enhance acquisition of P in low P soils in Africa and Latin America.

Introduction

Common bean (*Phaseolus vulgaris* L.) is among the priority legume crops in the world. It is the 2rd most produced crop in Latin America and the Caribbean (2.7 million ha). In Sub-Saharan Africa common bean is the 3rd most produced (5.8 million ha) after groundnut and cowpea (FAOSTAT, 2011; CGIAR, 2012). Common bean provides protein and minerals for millions of people in developing countries (CIAT, 2001, Broughton et al., 2003). Low phosphorus (P) availability is a primary constraint to bean production in many developing countries, affecting more than 70% of global production (Wortmann and Allen, 1994; Lynch, 1997; Wortmann et al., 1998; CIAT, 2001; Lynch, 2007). Phosphorus is relatively immobile in soil and it is mostly concentrated in the topsoil (Lynch and Brown, 2001; Lynch, 1995 and 2007). P availability is of particular concern in weathered and volcanic soils of the humid tropics and subtropics and in many sandy soils of the semiarid tropics, where yield is affected by lack of available inorganic P.

Fertilization to correct P deficiency has several important limitations in developing countries. The use of P fertilizers is often not efficient since P can be immobilized in the soil and became unavailable (Lambers et al., 2006, Simpson et al., 2011). Excessive fertilizers that are not used by plants can be removed by erosion, runoff and leaching. High concentrations of P in aquatic systems result in eutrophication and degradation of the environment (Cordell et al., 2009). In most developing countries, application of P fertilizer is low because it is expensive (World Bank, 2004, Borlaug, 2006, Lynch, 2007). The use of genetic improvement in crops to develop cultivars adapted to low P availability could be the best option (Lynch and Brown, 2012, Vance et al., 2003, Coudert et al., 2010, Rose et al., 2012).

Root traits are very important for crop adaptation to low P soils (Gahoonia et al., 1997; Ma et al., 2001a; Liao et al., 2001; Zhu and Lynch, 2004; Ochoa et al., 2006, Lynch, 2005). Plants display a wide range of adaptations to low P availability. Adaptation to low P environments is associated with root traits that enhance topsoil exploration, including shallow basal root growth angles (Bonser et al., 1996; Lynch and Brown 2001; Rubio et al., 2003; Ho et al., 2004; Zhu et al., 2005b; Lynch and Brown 2011), adventitious rooting (Miller et al., 2003;

Ochoa et al., 2006), lateral rooting (Zhu et al., 2005a), axial elongation (Borch et al., 1999), root hair length and density (Bates and Lynch, 1996, 2000, and 2001; Gahoonia et al., 1997; Miguel, 2004), aerenchyma formation (Fan et al., 2003) and reduced root respiration (Nielsen et al., 2001).

Interest in root architecture as a criterion for selection for crop adaptation to edaphic stresses has increased (Lynch and Brown, 2001; Vance et al., 2003; Lynch, 2007; Manschadi et al., 2008; Ramaekers et al., 2010; Coudert et al., 2010; Lynch and Brown 2012, Lynch, 2013, Rose et al., 2012). Genetic variability in root traits in different crops has been reported (Bonser et al., 1996; Gahoonia et al., 1997 and 2005; Miller et al., 2003; Rubio et al., 2003; Zhu and Lynch 2004; Zhu et al., 2005a and 2006; Burton, 2010; Bayuelo-Jiménez et al., 2010, Gahoonia and Nielsen, 2004). In maize, variation was reported for root architectural traits (Trachsel et al., 2010; Burton, 2010) and for root anatomical traits (Burton, 2010; Burton et al, 2012). Sarker et al. (2005) reported variation in taproot length and number of lateral roots in lentil. In beans, variation was reported for basal root whorl and basal root number (Widrig, 2005; Miguel, 2012), for root hair length and density (Yan et al., 1998, Miguel, 2004, Vieira et al., 2007), and for adventitious root number (Ochoa et al., 2006). Considering the large diversity of root traits, incorporation of root phenes into plant breeding programs would be useful for crop improvement.

Quantitative trait loci (QTL) controlling root traits have been reported in different crops. QTL have been identified in maize for root hair length associated with low and high P (Zhu et al., 2005c), lateral rooting (Zhu et al., 2005a), and seminal root length associated with low and high P (Zhu et al., 2006). Trachsel et al. (2009) identified QTL controlling root vigor and elongation rate of axile roots in maize. Wissuwa (1998) reported QTL controlling phosphorus-deficiency tolerance, Pup1, in rice. Gamuyao et al. (2012) showed that overexpression of PSTOL1, a candidate gene for phosphorus-starvation tolerance in rice varieties increased yield grain in soils deficient in P. The expression of PSTOL1 gene increases overall root growth by increasing the root length and root surface area. In beans, Liao et al. (2004) identified 16 QTL for root gravitropic traits (8 for shallow basal root length, 5 for relative shallow basal root length and 3 for basal root growth angle) and 6 controlling P uptake under low P conditions. Three of the QTL for gravitropic traits were associated with QTL for P uptake under low P, supporting the idea that root gravitropism contributes to P acquisition. Ochoa et al. (2006) identified two major QTL that controlled 61% of the variation in adventitious rooting in bean under low P conditions in the

field. Beebe et al. (2006) identified individual QTL controlling basal and tap roots. Their results showed that QTL controlling P accumulation coincided with basal root formation. These results suggest that basal roots are importance for P acquisition. Furthermore, Miguel (2012) identified 23 QTL associated with basal root whorl number (BRWN) and basal root number (BRN) in two bean populations, and his results were promising because some of the QTL were consistent across years. Seven of the ten QTL that he detected on DOR 364 x G 19833 population explained 7.4 to 23.8% of the variation in BRWN. A total of 13 QTL for BRN were detected in the same population, and individual QTL explained 13.7, 11.6, 9.4 and 9.2% of the variation in data from 2005, while 16.7 and 13.8% of the variation in BRN were explained by other two QTL in 2009. In another population (G 2333 x G 19839) one QTL was detected for BRWN and explained 19.4% of the phenotypic variation in data set from 2011. The control of root traits by QTL demonstrates that root traits are genetically controlled; therefore, root phenes could be targeted for crop improvement in breeding programs for development of varieties adapted to specific edaphic stresses. Since root traits are genetically controlled, selection for specific root traits to improved P acquisition could be the best alternative to improve crop productivity without the use of fertilizers. Marker Assisted Selection (MAS) could be used as an alternative for phenotypic root screening.

Root hairs play an important role in phosphorus uptake, especially in environments with low P availability. Root hairs are subcellular extensions of root epidermal cells. Root hair proliferation and elongation increase the volume of soil exploited by plants with low carbon cost. Several studies have reported that genotypes with long root hairs acquire more P (Gahoonia et al., 1997; Gahoonia and Nielsen, 1997; Yan and Lynch, 1998; Bates and Lynch, 2000; Wang et al., 2004, Zhu et al., 2005c). Bates and Lynch (2000) reported that root hairs are a cost efficient adaptation to low P after comparing Arabidopsis genotypes with and without root hairs. Gahoonia and Nielsen (1997) showed that among barley genotypes greater absorption of P was associated with longer and denser root hairs. P efficient genotypes with long and dense root hairs could be developed and deployed in regions with low P availability.

Wang et al., (2004) reported high heritability for root hair length from basal and tap roots and total roots; and low heritability for root hair density from basal and tap roots in soybean. Heritability of other root traits in common bean ranging from low to high were also reported (Araújo et al., 2005; Ochoa et al., 2006).

To improve bean yield in low-input agrosystems without the addition of fertilizers breeders need to identify and deploy cultivars with root systems suitable to the target region. The appropriate approach for variety improvement, particularly in developing countries of Africa and Latin America, where the use of molecular technologies for breeding is limited, could be selection through root phenotyping. Thus, genetic diversity and genetic basis of traits of interest in plant breeding need to be understood. The objectives of the present study were to develop bean populations from parents contrasting in root hair traits, to assess genetic variation in root hair length in two common bean populations, and to estimate the heritability of root hair length assessed on basal roots.

Materials and Methods

Plant material

Parental lines were selected based on results of root hair screening performed in 8-day old seedlings in the laboratory in 2006 (Table 4.1). Five different single crosses with parents contrasting in root hair traits were performed in the field at CIAT, Colombia in 2006, and for the present study we selected 2 populations, SEA 5 x SXB 418 and VAX 1 x SXB 418, because these populations had sufficient F3 seed and corresponding F4 lines for root hair evaluations. SEA 5 is a common bean genotype from Mesoamerican gene pool, with long and dense root hairs. VAX 1 is also a genotype from Mesoamerican gene pool with long and dense root hairs, while SXB 418 is from Mesoamerican gene pool but has short and sparse root hairs (Table 4.2). F2, F3 and F4 generations were developed in the field in Chokwe and Sussundenga, Mozambique from 2007 to 2009. Two hundred F3 plants from each cross were selected at random in 2008. Part of the seed of these F3 individual plants were advanced to generate F4 progeny families, and the remaining seeds were saved for further laboratory evaluation of root hair traits. SEA 5 x SXB 418 population was composed of 86 F3 individuals and corresponding 86 F4 progeny lines. VAX 1 x SXB 18 population was composed of 72 individuals of the F3 and 72 corresponding F4 progeny lines.

Laboratory experiment for evaluation of root hair traits

Four separate experiments were conducted to evaluate root hair traits of F3 and F4 generations of each population. In each experiment, progenies of each population were planted in a randomized complete block design (RCBD) in the laboratory in 2010 at Pennsylvania State University (PSU), USA. Parental lines from each cross were also included in each experiment. Each experiment consisted of 4 replications over time, and each experimental unit was composed of one plant. The same experimental design (RCBD) was used for F3 and F4 generations.

Seeds of F3 and F4 generations were surface-sterilized for 1-2 minutes with 10% NaOCl, rinsed with deionized water, mechanically scarified with a razor and germinated in rolls of brown germination paper No 78 (Anchor Paper Company, St. Paul, MN, USA). The rolls were placed upright in 5-liter beakers containing 1 L of 0.5 mM CaSO4. Seeds were placed in darkness at 28 ^{o}C for 3-4 days to allow germination. The seedlings were then placed in a plant culture room at 26 ^{o}C for 4 days with 12 hours of light. Roots were harvested 8 days after planting, and basal roots were separated from primary roots. Roots were separated from the shoots and stored in 50% ethanol for analysis of root hair traits.

Root hair imaging

For better visualization of root hairs, basal roots were briefly stained with dilute Toluidine blue O (0.05%). Root hair images were visualized with a light microscope (Nikon SMZ-4) and images were captured with a Nikon DS-Fi1 camera at 40x magnification and NIS-Elements F2.30 software. Additionally, an image of a hemacytometer (Hausser Scientific Horsham, PA, USA) was taken along with the root hair image for scale. Images were taken 2 cm from basal to the zone of emergence of new root hairs. Image J (http://rsbweb.nih.gov/ij/download.html) was used to measure root hair length. Root hair length and density of each line was measured in 5 different representative segments per replication. To assess the correlation between root hair length and density, root hair density was measured in a subset of 20 F4 lines from the two populations. The root hair density was measured by counting the number of root hairs in a representative known area. Root hair density was then converted to the number of root hairs per mm2 of root surface.

In preliminary experiments to identify parents contrasting in root hair traits we used a graduated hand magnifier to measure root hair length and to visually evaluate root hair density. DOR 364, known to have short and sparse root hairs and G19833, known to have long and dense root hairs, were used as control for comparison: The genotypes were then grouped in 3 root hair categories: Root hair length: short = less than 0.4 mm, intermediate = 0.4-0.5 mm, and long = greater than 0.5 mm. For root hair density the categories were sparse, intermediate and dense.

The remaining seed of F4 lines and other crosses were advanced to F5 and F6 generations for field evaluation of yield performance under low P stress, and for selection of P efficient lines adapted to Mozambique.

Data analysis

Data were analyzed using Minitab statistical software (2010 Minitab Inc., State College, PA, USA), and Statistix version 8 (Analytical Software, Tallahassee, FL, USA). Prior to analyses of variance, normality tests were performed to check if means observed in F3 and F4 generations of each population were normally distributed. Analyses of variance were performed separately for each of the 4 experiments as RCBD, and between families analysis of variance were also performed. Parental-offspring regression analyses were performed to estimate narrow sense (h^2) heritability of root hair length from basal roots in SEA 5 x SXB 418 and VAX 1 x SXB 418 populations.

Heritability of root hair length was estimated by calculating the regression coefficients between F4 progeny family means on F3 parental values:

Statistical model: $Y_i = b_o + bX_i + E_i$ (Fernandez and Miller, 1985)

Where:

Y_i = mean of progenies of i^{th} family

b_o = intercept

b = Regression coefficient

X_i = means of i^{th} single parent

E_i = Random errors, independent with normal distribution with mean of 0 and variance of σ^2.

The coefficient of regression ($b=h^2$) measures the proportion of parent-offspring covariance (Cov P-O) to the variance (Var) of the parent (σ_p^2): b = Cov P-O/σ_p^2. For the data in this study we computed (b) as b = CovF4-F3/Var F3.

Results

Genetic variation of root hair traits

Five common bean populations resulting from crosses of parents contrasting in root hair traits (Table 2) were developed: SEA 5 x SXB 418, VAX 1 x SXB 418, AFR 298 x PVA 773, Sel. 63 x SUG 41 and G 14665 x SUG 41. Each population was composed by 200 lines at F4 generation. For detection of genetic variation and heritability of root hair length two populations were used: SEA 5 x SXB 418 and VAX 1 x SXB 418. Selected lines and progenies from the remaining crosses were advanced for future studies and field evaluations of yield performance under low P conditions.

Considerable variation in root hair length from basal roots was found among F3 individuals and among F4 lines in SEA 5 x SXB 418 and VAX 1 x SXB 418 populations (Figure 4.1 and Figure 4.2). The normality test performed separately in F3 and F4 lines in the two populations indicated that the means of the samples were normally distributed, thus, the data from the present study were not transformed.

The differences in root hair length between the two populations in F3 and F4 generations were significant ($p < 0.001$) (Table 4.3), and significant differences in root hair length among individuals within populations was detected at $p < 0.001$ in both populations (Table 4.4). The average root hair length for the SEA 5 x SXB 418 population varied from 0.19 to 0.77 mm in the F3 generation, and from 0.197 to 0.784 mm in the F4 (Table 4. 5). For the VAX 1 x SXB 418 population, the root hair length in the F3 generation varied from 0.20 to 0.916 mm, and similar

trend was observed in the F4 generation, where the root hair length varied from 0.187 to 0.843 mm (Table 4.5).

Based on root hair length, we grouped the F4 lines into three categories corresponding to short (less than 0.4 mm), intermediate (0.4 - 0.5 mm) and long root hairs (more than 0.5 mm). In the SEA 5 x SXB 418 population, 21.4 % of the 84 F4 lines had long root hairs, 35.7 % had intermediate, and 42.9 % had short root hairs, while the VAX 1 x SXB 418 population had 36.6% of the 72 F4 lines with long root hairs, 30.9 % with intermediate, and 32.5 % with short root hairs.

Root hair density (number of root hairs per square mm) was measured in a subset of 20 F4 lines from each population to determine the correlation between root hair length and density on basal roots. The average number of root hairs per square mm varied from 102 to 275 in the SEA 5 x SXB 418 population, and from 92 to 307 in the VAX 1 x SXB 418 population. Root hair length and density were positively and strongly correlated in the two populations. The coefficients of determination (R^2) were 0.72 ($p < 0.001$) for the SEA 5 x SXB 418 population, and 0.68 ($p<0.001$) for the VAX 1 x SXB 418 population (Figures 4.3 and 4.4).

Heritability of root hair length

Estimates of narrow-sense heritability of root hair length from basal roots evaluated in 8 day old bean seedlings were based on regression coefficients ($b= h^2$) of F4 values of offspring on F3 values of parents. The estimated heritability of root hair length from basal roots measured in 8 day old bean seedlings in the two populations under laboratory conditions was moderately high. The estimated h^2 was 0.69 for SEA 5 x SXB 418 population and 0.71 for VAX 1 x SXB 418 population (Table 4.6). Moderately high and positive correlations were found between F3 parents and F4 progenies in the two populations (Figures 4.5 and 4.6). Coefficients of determination (R^2) obtained from correlations between F4 progenies and F3 parent means were 0.51 $p < 0.001$) and 0.61 ($p < 0.001$) for the SEA 5 x SXB 418 and VAX 1 x SXB 418 populations, respectively (Table 4.6), indicating that more than half the variation in root hair length in the F4 progenies was due to variation in root hair length in the F3 parents.

Discussion

Root hairs play an important role in P uptake, especially in environments with low P availability. In this study, we report large genetic variation in root hair length assessed on basal roots of F3 and F4 lines in two bean populations. Moreover, significant differences in root hair length between the two families developed in the present study were evident. The two populations were derived from parents contrasting in root hair traits, one with longer and denser root hairs and the other with shorter and sparser root hairs; thus, we expected to find genetic variation among F3 and F4 lines derived from these parents. Variation in root hair density was also detected among F4 lines in both populations. The genetic variation in root hair length and density found in this study and previously reported variation in other root traits (Lynch and Beebe, 1995; Miller et al., 2003; Ochoa et al., 2006, Bonser et al., 1996; Gahoonia et al., 1997 and 2005; Rubio et al., 2003; Zhu and Lynch 2004; Zhu et al., 2005a and 2006; Burton, 2010; Bayuelo-Jiménez et al., 2010; Lynch, 2011) may be associated with plant adaptation to different agroecological conditions where crops evolved or were selected by farmers. In the case of common bean, differences in root adaptation may be associated with agroecological conditions from Mesoamerica and Andean regions.

Although we used only two populations, our results indicate that large genetic variation in root hair length and density can be generated among lines resulting from crosses of parents contrasting in root hair traits. We found high correlation between root hair length and density in the two population used in the present study, and similar results were reported in Chapter 3 of this dissertation. Since evaluation of root hair density requires more time compared to evaluation of root hair length, direct selection of genotypes with longer root hairs can also be used to select for denser root hairs. Root hair length and density traits have been shown to have synergistic effects on P uptake (Ma et al, 2001b), so it is not surprising that they would be correlated.

The bean lines used in the present study were developed from parents contrasting in root hair length and density; that is, contrasting in traits for low P stress adaptation. SEA 5 had average root hair length of 0.66 mm, VAX 1 had root hair length of 0.81 mm, while SXB 418 had root hair length of 0.225 mm on average. The progenies resulting from these crosses showed substantial variation in root hair length and density. We observed that some progenies in both populations had root hairs that were greater or less than the means for the parents, indicating

transgressive segregation. The presence of transgressive segregation in these populations may suggest that each parent carries alleles that both increase and decrease root hair length. Similar results were found for root hair density. It should therefore be possible to select superior root hair traits in segregating populations. In addition, the large genetic variation in root hair traits found in this study and also reported in previous studies indicate that direct selection by root hair phenotyping could be useful for genetic improvement of crops that will result in increased yields in regions with low P stress.

One of the methods used to determine heritability of traits in plants is parent - progeny regression. This procedure involves the regressing of the mean value of a trait in the progeny upon the value of the same trait in the parent (Frey and Horner, 1955 and 1957; Smith and Kinman, 1965; Fernandez and Miller, 1985), and it estimates the narrow sense heritability of a trait. Heritability in a narrow sense is important to plant breeders because the gain from selection depends on the additive portion of genetic variance in relation to the total variance (Fernandez and Miller, 1985). The relatively large parent-offspring regression coefficient obtained in both common bean populations under laboratory conditions in the present study indicates that root hair length has moderately high heritability. Similar results were reported in soybean by Wang et al., 2004, who found heritability of 57.85 % and 59.18% in root hair length from basal and tap roots, respectively. In beans, Araújo et al. (2005) reported high to moderate broad-sense heritability for root area, root length and root mass, and P content in beans. Narrow-sense heritability ranging from low (0.25) to high (0.51) was detected for adventitious root traits under low and high P (Ochoa et al., 2006). These findings reinforcing the idea that genetic improvement of root traits could be a better option for developing varieties adapted to low P environments. Knowledge about the heritability of root hair length could help breeders to develop better strategies for genetic improvement of cultivars adapted to low P stresses.

The results we found in the present study are promising for bean breeding. Although root hairs are highly affected by the environment, the moderately high heritability observed in the two populations used in the present study indicates that genetic improvement by conventional breeding could be effective for development of genotypes with longer and denser root hairs. Direct phenotyping and conventional hybridization followed by selection under low P stress could result in deployment of cultivars with longer and denser root hairs. However, conventional breeding could also transfer undesirable traits that are passed on from parents to progenies. One

alternative to minimize the inheritance of undesirable traits is the use of back cross method or molecular markers. Marker assisted selection could be used for phenotypic selection of genotypes with desirable root hair phenes. Information on QTL controlling different root phenes would help in developing cultivars with multiple mechanisms that enhance P acquisition. We found that root hair length is heritable when evaluated in early generations and early stages of the plants (8 day old seedlings) under laboratory conditions. The high heritability suggests that considerable progress may be expected from selection for longer and denser root hairs in segregating bean populations.

To follow up the results of the present study, the segregating F6 lines advanced from different crosses are being tested for yield performance under low P stress in Mozambique to check the utility of longer and denser root traits over shorter and sparser root hairs. Simultaneously we are selecting P efficient lines adapted to different agro-ecological conditions of Mozambique.

Conclusions

Large genetic variation in root hair length and density from basal roots within F3 and within F4 generations was found, and significant variation in root hair length between bean populations was detected. Between and within family variation in root hair length was also detected. Information on genetic diversity of root hair traits is important in breeding programs for development of varieties adapted to low P stress. Breeding for longer and denser root hairs and other root traits could enhance acquisition of P in low P soils of developing countries. We were able to estimate the heritability of root hair length from basal roots on common bean using parent-offspring regression method in two bean populations. The moderately high heritability of root hair length from basal roots found in the present study implies that breeders could select for P efficient common bean lines based on root hair length. Our results provide confidence that reasonable selection progress for long root hairs could be expected on segregating progenies derived from parents used in the present study.

The significance of the information on root hair trait heritability, with QTL of different traits could be important for breeding program for development of P efficient beans. Additional

research to understand better the genetic basis of the root hair traits will be beneficial for development of cultivars with superior yields in regions with low P stress.

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Table 4-1. List of common bean genotypes and their root phenotypes identified during screening of parents. Root hair traits were measured separately on primary and basal roots. Basal root whorl number (BRWN), basal root number (BRN), root hair length (RHL), root hair density (RHD). Root hair category: Length: Short (< 0.4 mm), Intermediate (Inter.) (0.4-0.5 mm), Long (> 0.5 mm). Density: Sparse, intermediate and dense. The RHD was based on visual evaluations, where DOR 364 (with sparse root hairs) and G19833 (with dense root hairs) were used for comparison. The data are means of 4 replications.

Andean gene pool

		Basal root			Primary root				
Order #	Genotype	RHL (mm)	RHD	Category	RHL (mm)	RHD	Category	BRWH	BRN
1	BRB217	0.53	Dense	Long/Dense	0.595	Dense	Long/Dense	3.0	11.75
2	G4017	0.78	Dense	Long/Dense	0.795	Dense	Long/Dense	2.0	7.5
3	SUG47	0.38	Sparse	Short/Sparse	0.4	Sparse	Short/Sparse	2.75	11.75
4	G14665	0.65	Dense	Long/Dense	0.795	Dense	Long/Dense	3.5	13.25
5	SEQ1005	0.62	Dense	Long/Dense	0.73	Dense	Long/Dense	2.25	8.75
6	SEQ1001	0.48	Inter	Inter	0.585	Dense	Long/Dense	2.25	10.0
7	SEQ1006	0.365	Sparse	Short/Sparse	0.305	Sparse	Short/Sparse	2.75	10.5
8	AFR298	0.705	Dense	Long/Dense	0.73	Dense	Long/Dense	2.0	6.5
9	DRK 156	0.365	Sparse	Short/Sparse	0.485	Inter	Inter	1.75	5.25
10	RAA18	0.61	Dense	Long/Dense	0.655	Dense	Long/Dense	2.75	9.0
11	RAA19	0.66	Dense	Long/Dense	0.76	Dense	Long/Dense	2.0	8.5
12	RAA30	0.685	Dense	Long/Dense	0.725	Dense	Long/Dense	2.0	5.5
13	BRB25	0.725	Dense	Long/Dense	0.785	Dense	Long/Dense	2.0	6.5
14	AND277	0.3	Sparse	Short/Sparse	0.42	Inter	Inter	3.0	9.75
15	G122	0.41	Inter	Inter	0.46	Inter	Inter	3.0	9.5
16	SEQ1039	0.31	Sparse	Short/Sparse	0.475	Inter	Inter	2.5	9.0
17	BRB156	0.465	Inter	Inter	0.58	Inter	Long/Inter	2.75	8.75
18	BRB211	0.365	Sparse	Short/Sparse	0.44	Inter	Inter	2.5	10.0
19	AFR640	0.51	Dense	Long/Dense	0.59	Dense	Long/Dense	2.0	7.0
20	G23823E	0.705	Dense	Long/Dense	0.79	Dense	Long/Dense	3.0	12.0
21	AFR663	0.365	Sparse	Short/Sparse	0.48	Inter	Inter	2.0	8.0
22	G17722	0.73	Dense	Long/Dense	0.76	Dense	Long/Dense	2.25	8.75
23	RAA20	0.575	Dense	Long/Dense	0.635	Dense	Long/Dense	2.0	7.25
24	BRB183	0.61	Dense	Long/Dense	0.775	Dense	Long/Dense	2.0	7.5

Mesoamerican gene pool

		Basal root			Primary root				
Order #	Genotype	RHL (mm)	RHD	category	RHL (mm)	RHD	category	BRWN	BRN
1	DOR 390	0.42	Sparse	Inter	0.425	Sparse	Short/Sparse	2.0	8.0
2	SEA 15	0.55	Dense	Long/Dense	0.695	Dense	Long/Dense	1.0	4.0
3	SEQ 11	0.57	Inter	Long/Sparse	0.665	Inter	Long/Inter	2.0	7.0
4	SEA 5	0.69	Dense	Long/Dense	0.745	Dense	Long/Dense	2.0	7.25
5	G 19833	0.645	Dense	Long/Dense	0.57	Dense	Long/Dense	2.0	8.25
6	TIO CANELA	0.385	Inter	Inter	0.545	Inter	Long/Inter	2.0	8.0
7	CAL 143	0.545	Inter	Inter	0.58	Inter	Long/Inter	3.0	10.0
8	DOR 364	0.400	Sparse	Short/Sparse	0.410	Sparse	Short/Sparse	2.0	8.0
9	PINTO VILLA	0.735	Dense	Long/Dense	0.72	Dense	Long/Dense	2.0	8.0
10	SEQ 1003	0.475	Inter	Inter	0.54	Inter	Long/Inter	2.0	8.5
11	PVA 773	0.295	Sparse	Short/Sparse	0.405	Sparse	Short/Sparse	3.0	10.0
12	G 2333	0.71	Dense	Long/Dense	0.86	Dense	Long/Dense	2.0	8.0
13	BAT 477	0.565	Dense	Long/Dense	0.645	Dense	Long/Dense	2.0	7.75
14	G 21212	0.815	Dense	Long/Dense	0.795	Dense	Long/Dense	2.0	8.0
15	VAX 1	0.67	Dense	Long/Dense	0.735	Dense	Long/Dense	2.0	8.0
16	SER 16	0.585	Inter	Inter	0.625	Inter	Long/Inter	2.0	7.5
17	SAB 258	0.6	Inter	Inter	0.75	Dense	Long/Dense	2.0	7.5
18	A 774	0.625	Dense	Long/Dense	0.685	Dense	Long/Dense	2.0	8.5
19	SER 118	0.455	Inter	Inter	0.54	Inter	Long/Inter	2.0	7.75
20	CARIOCA	0.652	Dense	Long/Dense	0.75	Dense	Long/Dense	2.0	8.0
21	SXB 412	0.69	Dense	Long/Dense	0.75	Dense	Long/Dense	1.75	7.0
22	SXB 418	0.425	Sparse	Inter/Sparse	0.33	Sparse	Short/Sparse	2.0	7.75
23	RAB 655	0.595	Inter.	Long/Inter.	0.685	Dense	Long/Dense	1.5	6.5
24	A 286	0.55	Inter	Long/Inter	0.55	Inter	Long/Inter	2.0	8.25
25	NCB 226	0.495	Inter	Inter	0.54	Inter	Long/Inter	2.0	7.5
26	G 4523 (Ica P.)	0.355	Inter	Short/Inter	0.48	Inter	Inter	2.0	7.75
27	San Cristobal	0.53	Inter	Long/Inter	0.595	Inter	Long/Inter	2.0	7.75

Table 4-2. Description of root traits of bean genotypes used to perform 5 single crosses. AFR 298, G 14665, Selection 63 crema, SEA 5 and VAX 1 have long and dense root hairs, and PVA 773, SXB 418, and SUG 47 have short and sparse root hairs. Basal root whorl number (BRWN), Basal root number (BRN), Root hair length (RHL), Root hair density (RHD). Root hair category: Root hair length: Short: less than 0.4 mm, Intermediate = 0.4-0.5 mm, Long = greater than 0.5 mm. Root hair density: Sparse and Dense. The root hair density was based on visual evaluations, where DOR 364 (known to have sparse root hairs) and G19833 (with dense root hairs) were used as control for comparison.

Parent line[1)]	Gene pool			Basal root		Primary root		Root hair category
		BRWN	BRN	RHL (mm)	RHD	RHL (mm)	RHD	
AFR 298	Andean	2	6.5	0.71	Dense	0.73	Dense	Long/Dense
G 14665	Andean	3.5	13.25	0.65	Dense	0.79	Dense	Long/Dense
Sel. 63	Andean	2.5	8	0.71	Dense	0.69	Dense	Long/Dense
PVA 773	Andean	3	10	0.29	Sparse	0.4	Sparse	Short/Sparse
SUG 47	Andean	2.75	11.75	0.38	Sparse	0.4	Sparse	Short/Sparse
SEA 5	Mesoamerican	2	7.25	0.69	Dense	0.75	Dense	Long/Dense
VAX 1	Mesoamerican	2	8	0.67	Dense	0.74	Dense	Long/Dense
SXB 418	Mesoamerican	2	7.75	0.42	Sparse	0.33	Sparse	Short/Sparse

[1)] Single crosses performed with parents contrasting in root hair traits: SEA 5 x SXB 418, VAX 1 x SXB 418, AFR 298 x PVA 773, Sel. 63 x SUG 41 and G 14665 x SUG 41.

Table 4-3. Analyses of variance across populations for F3 and F4 generations. Data are means of 4 replications. SEA 5 x SXB 418 population was composed of 86 F3 and F4 lines, and the two parents. VAX 1 x SXB 418 population was composed of 73 F3 and F4 lines including the two parents. DF = Degrees of freedom. ** significant at $p \leq 0.01$, *** significant at $p < 0.001$.

	DF	P value
Source of variance	F3 generation	
Between families	1	0.001***
Within families	157	< 0.000***
	F4 generation	
Between families	1	0.01**
Within families	157	< 0.000***

Table 4-4. Analyses of variance of root hair length for F3 and F4 generations of each population evaluated in 8 day old bean seedlings. The SEA 5 x SXB 418 population included 86 F3 and F4 lines including the two parents and, the VAX 1 x SXB 418 population was composed of 73 F3 and F4 lines including two parents. DF = Degrees of freedom. *** - significant at $P \leq 0.001$.

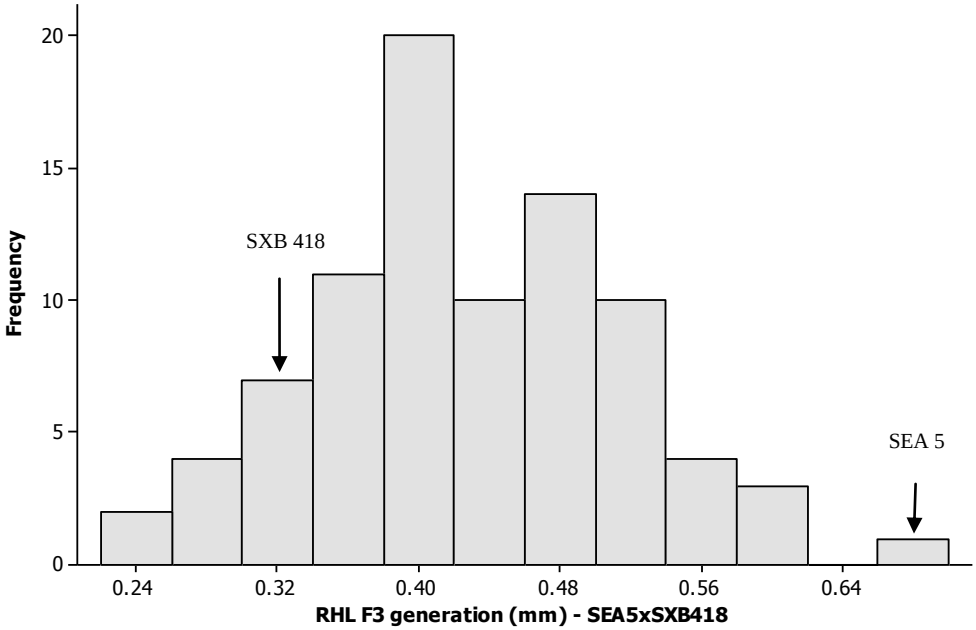
Source of variance	DF	MS	P value
		SEA 5 x SXB 418	
F3 progeny	85	0.030	< 0.0001***
F4 progeny	85	0.028	< 0.0001***
		VAX 1 x SXB 418	
F3 progeny	73	0.065	< 0.0001***
F4 progeny	73	0.053	< 0.0001***

Table 4-5. Summary statistics of root hair length measured in 8 day old bean seedlings from two populations SEA 5 x SXB 418 and VAX 1 x SXB 418. The data in each generation are average of 4 replications. RHL = Root hair length (mm), CV = Coefficient of variance. P1 and P2 = parent 1 with longer and denser root hairs and parent 2 with shorter and sparser root hairs.

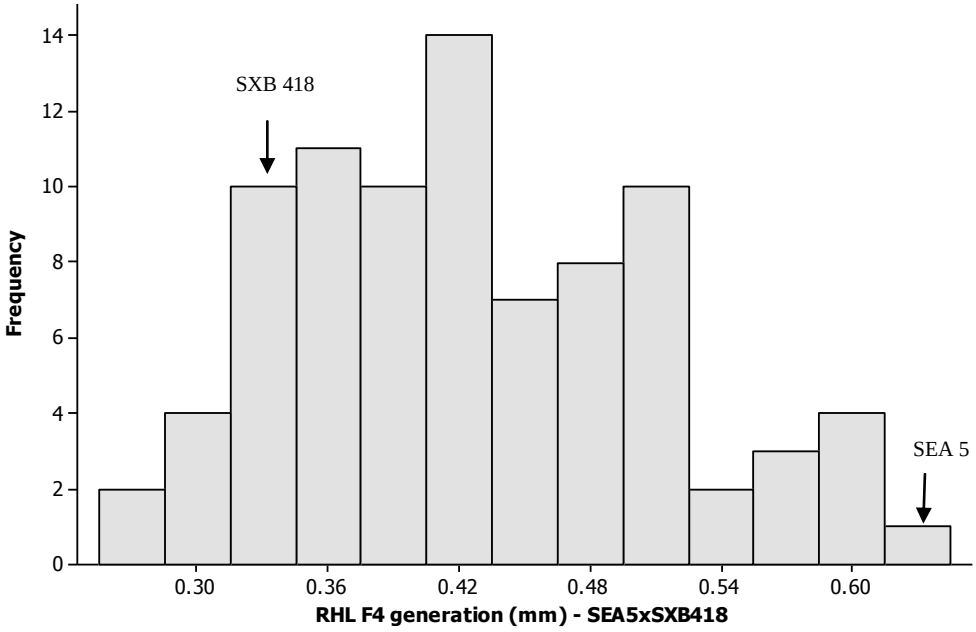
	RHL (mm)			
Generation	Mean ±SE	Minimum	Maximum	CV (%)
SEA 5 x SXB 418				
F3	0.426 ± 0.005	0.191	0.773	25.43
F4	0.427 ± 0.006	0.197	0.784	24.42
P1 (SEA 5)	0.662 ± 0.032	0.594	0.745	9.631
P2 (SXB 418)	0.345 ± 0.017	0.294	0.374	10.25
VAX 1 x SXB 418				
F3	0.485 ± .008	0.209	0.916	28.78
F4	0.467 ± 0.007	0.237	0.843	27.61
P1 (VAX 1)	0.809 ±0.013	0.782	0.843	3.22
P2 (SXB 418)	0.225 ±0.223	0.187	0.265	18.27

Table 4-6. Estimates of heritability of root hair length by parent-offspring regression method ($b = h^2$) (± SE) between F4 progeny family means and parental F3 values, and coefficients of determination in two common bean populations. The levels of significance for regression analyses are presented. *** Significant at $p < 0.001$.

Population	n	*b*	R^2
SEA 5 x SXB 418	86	0.69 ± 0.073***	0.51***
VAX 1 x SXB 418	73	0.71 ± 0.066)***	0.61***

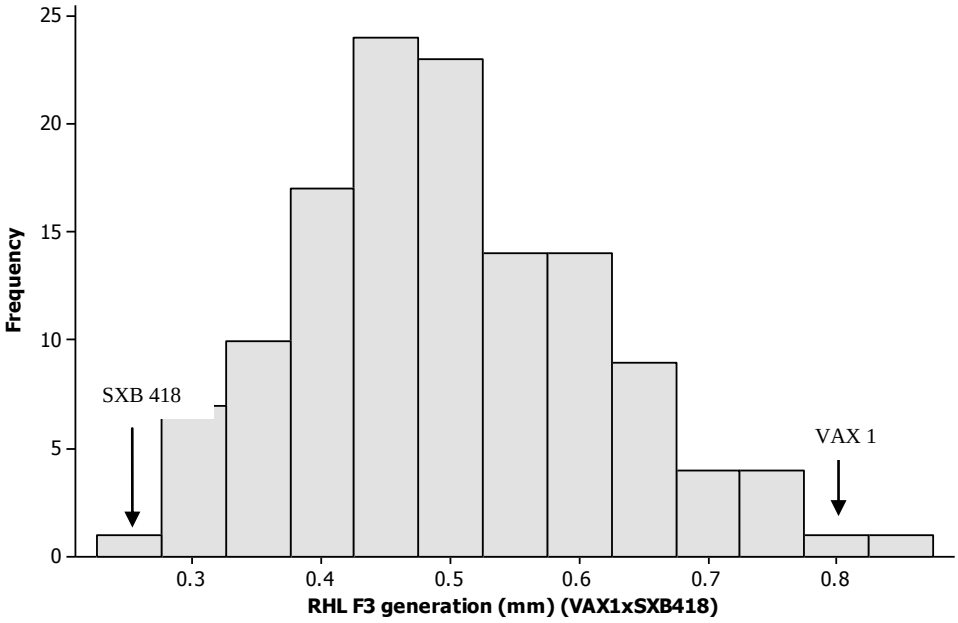


a) F3 generation

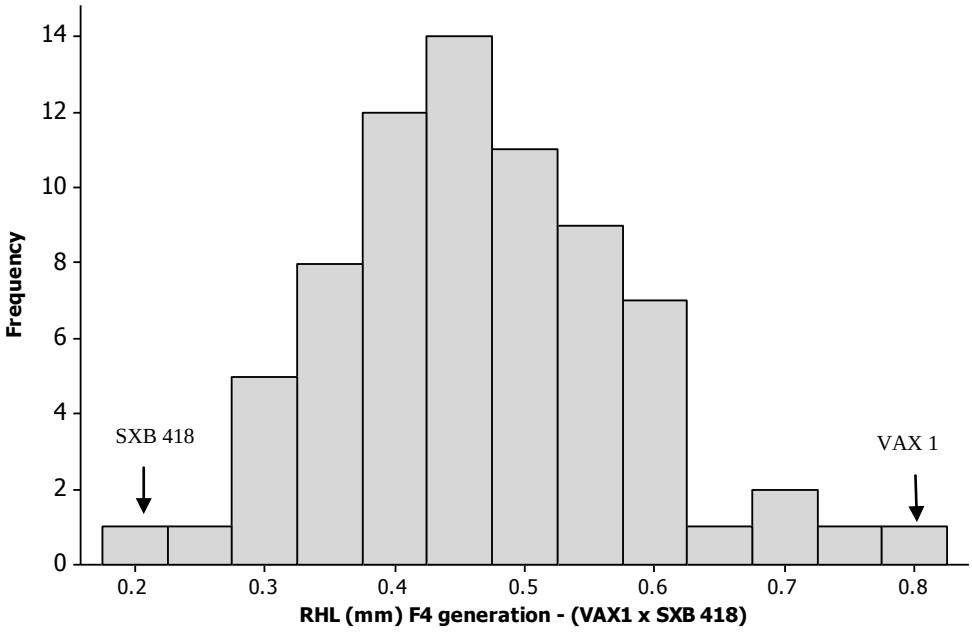


b) F4 generation

Figure 4-1. Phenotypic distribution of root hair length from basal roots of 86 F3 individual plants (a) and 86 F4 progeny (b) from SEA 5 x SXB 418 populations measured in 8 day old bean seedlings. The results are means of 4 replications.



a) F3 generation



b) F4 generation

Figure 4-2. Phenotypic distribution of root hair length of 73 F3 individual plants (a) and 73 F4 progeny (b) from VAX 1 x SXB 418 population measured in 8 day old bean seedlings. The data are means of 4 replications.

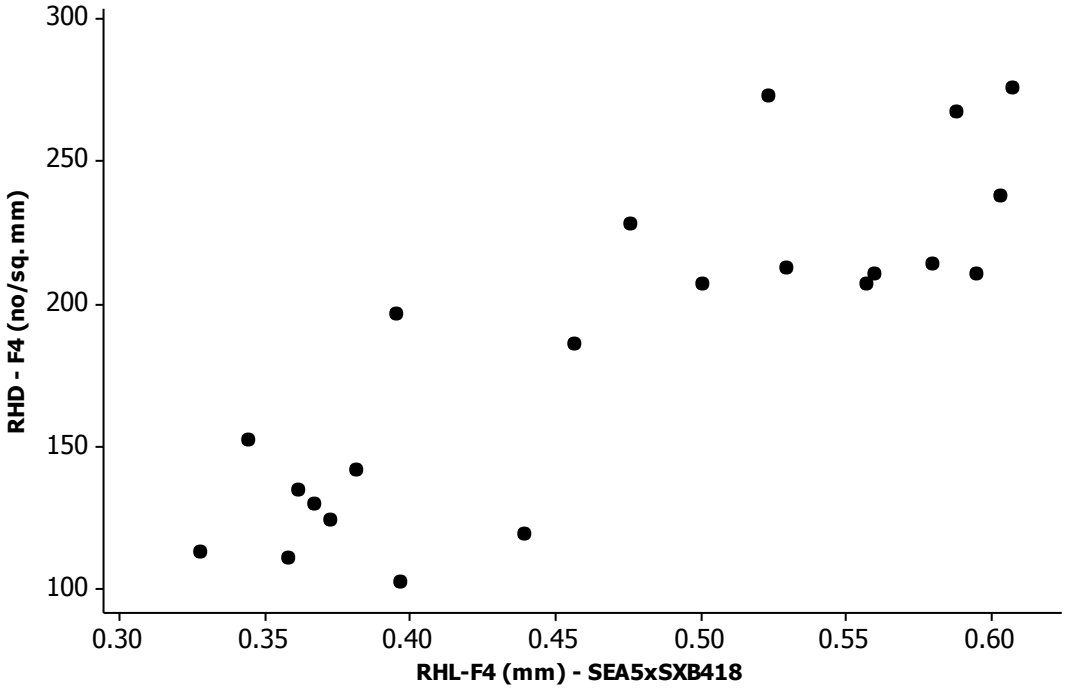


Figure 4-3. Scatterplot showing positive correlation between root hair length (RHL) and root length density (RHD) on selected F4 lines from SEA 5 x SXB 418 population measured in 8 day old common bean seedlings. Each point represents an average of 4 replications. $R^2 = 0.72$, $P < 0.001$.

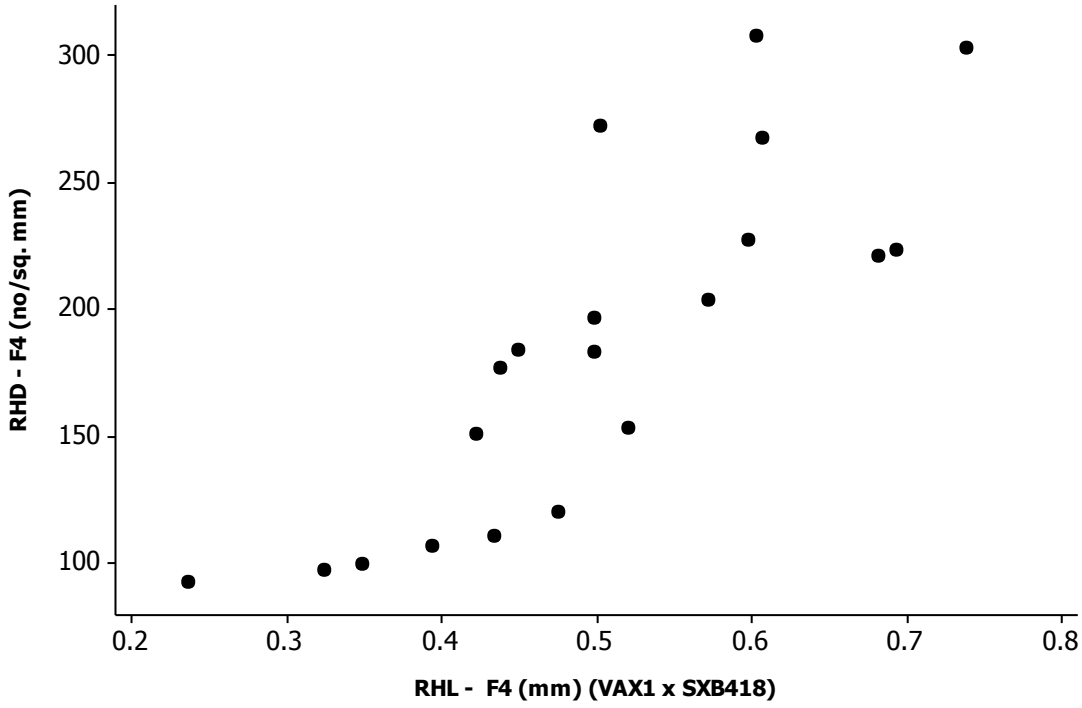


Figure 4-4.Scatterplot showing positive correlation between root hair length (RHL) and root length density (RHD) on selected F4 lines from VAX 1 x SXB 418 population measured in 8 day old common bean seedlings. Each point represents an average of 4 replications. $R^2 = 0.68$, $P < 0.001$.

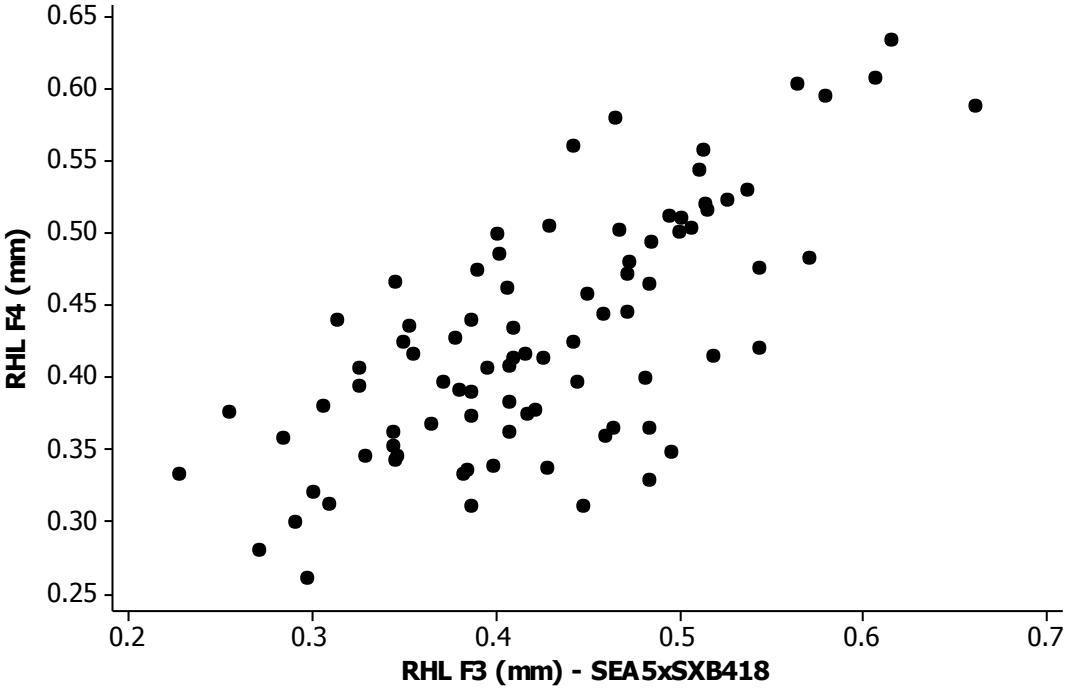


Figure 4-5.Scatterplot showing positive correlation between root hair lengths from basal roots measured on F4 progeny and F3 parents from SEA 5 x SXB 418 population. Each point represents an average of 4 replications. RHL = Root hair length. $R^2 = 0.51$, $P < 0.001$.

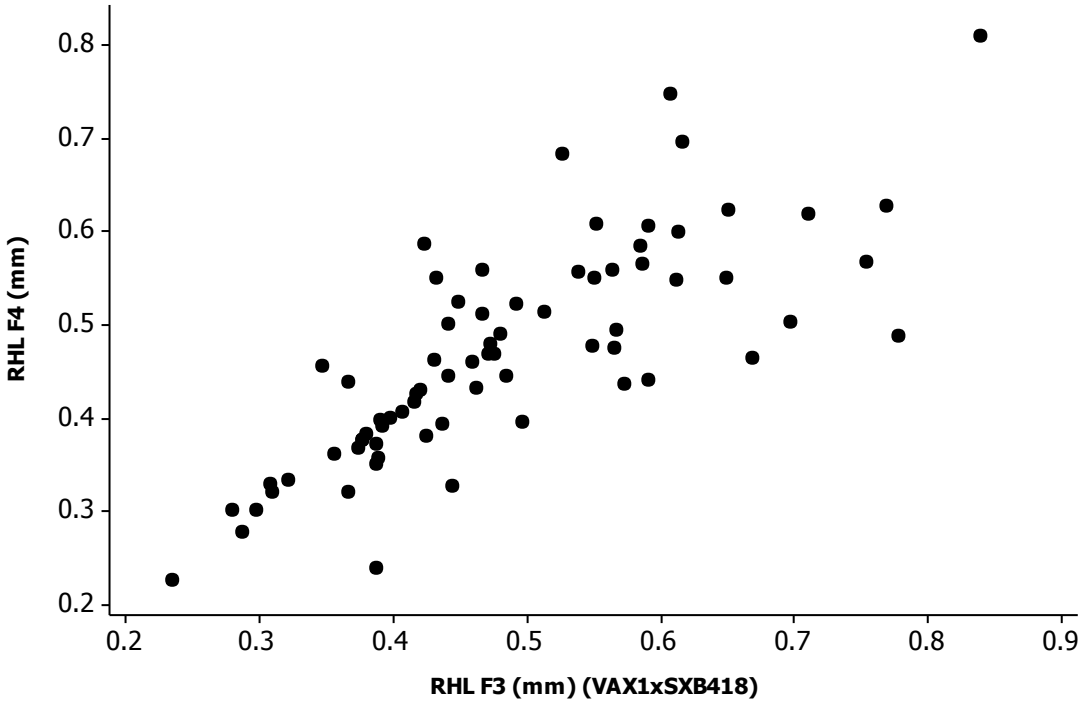


Figure 4-6. Scatterplot showing positive correlation between root hair lengths from basal roots measured on F4 progeny and F3 parents from VAX 1 x SXB 418 population. Each point represents an average of 4 replications. RHL = Root hair length. $R^2 = 0.61$, $P < 0.001$.

Chapter 2 Appendix

Appendix 2-1: List of 64 common bean genotypes evaluated for basal root whorl number and basal root number in the laboratory at Penn State University. The genotypes were provided by the International Center for Tropical Agriculture (CIAT) and few were from the Agriculture Research Institute of Mozambique (IIAM).

Genotype	Gene pool	Source
A 286	Mesoamerican	CIAT
A 774	Mesoamerican	CIAT
AFR 708	Andean	CIAT
AFR 298	Andean	CIAT
AFR 640	Andean	CIAT
AFR 663	Andean	CIAT
AND 277	Andean	CIAT
BAT 477	Mesoamerican	CIAT
Bonus	Andean	IIAM
BRB 156	Andean	CIAT
BRB 183	Andean	CIAT
BRB 211	Andean	CIAT
BRB 217	Andean	CIAT
BRB 25	Andean	CIAT
CAL 96	Andean	CIAT
CAL143	Andean	CIAT
Carioca	Mesoamerican	CIAT
Diacol Calima	Andean	IIAM
Doctor	Andean	IIAM
DOR 500	Mesoamerican	CIAT
DOR 364	Mesoamerican	CIAT
DOR 390	Mesoamerican	CIAT
DRK 156	Andean	CIAT
DRK 16	Andean	CIAT
G 19833	Andean	CIAT
G 122	Andean	CIAT
G14665	Andean	CIAT
G 17722	Andean	CIAT
G 21212	Mesoamerican	CIAT
G 2333	Mesoamerican	CIAT
G 23823E	Mesoamerican	CIAT

G 4017	Andean	CIAT
G 4523	Andean	CIAT
Ica Pijão	Mesoamerican	IIAM
LIC-04-1-3	Andean	IIAM
LIC-04-2-4	Andean	IIAM
LIC-04-3-4	Andean	IIAM
LIC-04-5-2	Andean	IIAM
LIC-04-9-4	Andean	IIAM
NCB 226	Mesoamerican	CIAT
Pinto Villa	Mesoamerican	CIAT
PVA 773	Andean	CIAT
RAA 18	Andean	CIAT
RAA 19	Andean	CIAT
RAA 20	Andean	CIAT
RAA 30	Andean	CIAT
RAB 655	Mesoamerican	CIAT
SAB 258	Andean	CIAT
San Cristob.	Mesoamerican	CIAT
SEA 15	Mesoamerican	CIAT
SEA 5	Mesoamerican	CIAT
SEQ 1001	Andean	CIAT
SEQ 1003	Andean	CIAT
SEQ 1005	Andean	CIAT
SEQ 1006	Andean	CIAT
SEQ 1039	Andean	CIAT
SEQ 11	Andean	CIAT
SER 16	Mesoamerican	CIAT
SER 118	Mesoamerican	CIAT
SUG 47	Andean	CIAT
SXB 412	Mesoamerican	CIAT
SXB 418	Mesoamerican	CIAT
Tio Canela	Mesoamerican	CIAT
VAX 1	Mesoamerican	CIAT

Appendix 2-2: List of 30 common bean genotypes used for field evaluations in Chokwe and Umbeluzi, Mozambique in 2008 and 2009 (subset from the 64 genotypes evaluated in the laboratory). The basal root whorl number and basal root number of these genotypes were also evaluated in the laboratory. na – information not available

Genotype	Source	Gene pool
CAL 96	CIAT	Andean
VAX 1	CIAT	Mesoamerican
SEA 15	CIAT	Mesoamerican
SEA 5	CIAT	Mesoamerican
PVA 773	CIAT	Andean
Tio Canela	CIAT	Mesoamerican
CAL 143	CIAT	Andean
G 19833	CIAT	Andean
DOR 364	CIAT	Mesoamerican
Doctor	IIAM	Andean
LIC-04-1-3	IIAM	Andean
DRK 16	CIAT	Andean
SEQ 1003	CIAT	Andean
BAT 477	CIAT	Mesoamerican
SXB 418	CIAT	Mesoamerican
SER 16	CIAT	Mesoamerican
Bonus	IIAM	Andean
SAB 258	CIAT	Andean
A 774	CIAT	Mesoamerican
G 2333	CIAT	Mesoamerican
Diacol Calima	IIAM	Andean
AFR 708	IIAM	Andean
Carioca	CIAT	Mesoamerican
G 4523 (IP)	CIAT	Andean
G 21212	CIAT	Mesoamerican
SXB 412	CIAT	Mesoamerican
RAB 655	CIAT	na
DOR 500	CIAT	Mesoamerican
AFR 298	CIAT	Andean
Ica Pijão	IIAM	Mesoamerican

Appendix 2-3: List of 20 genotypes from the common bean core collection evaluated under low phosphorus in Rock Springs, Pennsylvania, USA in 2010. All the genotypes were provided by CIAT. Races: NG1 - Nueva Granada, group 1, NG2 - Nueva Granada, group 2, P1 – Peru, group 1, M1 – Mesoamericana, group 1, G – Guatemala, D1 – Durango, group 1 and D2 – Durango, group 2. na – not available data.

Genotype	Gene pool	Race	Origin
AND 1005.	Andean	NG1	Colombia
BAT 477.	Mesoamerican	na	Colombia
DOR 364.	Mesoamerican	NG2	El Salvador
DRK 47.	Andean	P1	Colombia
G 738.	Andean	NG1	Guatemala
G 1328.	Mesoamerican	M1	Mexico
G 1797.	Mesoamerican	G	Mexico
G 2567.	Andean	P1	Ecuador
G 2686.	Andean	P1	Peru
G 3807.	Mesoamerican	M1	Brazil
G 3936.	Mesoamerican	D2	Costa Rica
G 4258.	Mesoamerican	NG2	Guatemala
G 4278.	Mesoamerican	M2	Mexico
G 4494.	Mesoamerican	D2	Mexico
G 7742.	Mesoamerican	D1	Mexico
G 12806.	Mesoamerican	D2	Mexico
G 18147.	Mesoamerican	M2	Haiti
G 19833.	Andean	P1	Peru
G 19848.	Andean	NG2	Peru
G 22044.	Mesoamerican	D1	Mexico

Appendix 2-4: Mean separation (Tukey test) of visual scores of 30 genotypes evaluated in Chokwe, Mozambique. Means with the same letter are not statistically different at 5% level of significance. Traits that the differences among genotypes were not statistically significant are not presented. The data are average of two years (2008 and 2009) and 4 replications.

1) Adventitious root branching

Tukey HSD All-Pairwise Comparisons Test of Adventitious root branching for Genotype

Genotype	Mean	Homogeneous Groups
G 2333	3.7500	A
LIC-04-1-3	3.2500	AB
RAB 655	3.2500	AB
AFR 298	3.1250	AB
DOR 500	3.0000	AB
G 19833	3.0000	AB
SXB 418	3.0000	AB
VAX 1	3.0000	AB
Carioca	3.0000	AB
SER 16	2.8750	AB
G 21212	2.8750	AB
PVA 773	2.8750	AB
BAT 477	2.7500	AB
DOR 364	2.7500	AB
ICA PIJAO	2.7500	AB
SEA 5	2.7500	AB
BONUS	2.6250	AB
DRK 16	2.6250	AB
SXB 412	2.6250	AB
AFR 708	2.6250	AB
CAL 96	2.6250	AB
A 774	2.5000	B
CAL 143	2.5000	B
D.CALIMA	2.5000	B
DOCTOR	2.5000	B
G 4523 (IP)	2.3750	B
SAB 258	2.3750	B
SEQ 1003	2.3750	B
T.CANELA	2.3750	B
SEA 15	2.1250	B

Alpha 0.05 Standard Error for Comparison 0.3300
Critical Q Value 5.299 Critical Value for Comparison 1.2366
Error term used: Year*Rep*Genotype, 177 DF
There are 2 groups (A and B) in which the means are not significantly different from one another.

2) Adventitious root number

Tukey HSD All-Pairwise Comparisons Test of Adventitious root number for Genotype

Genotype	Mean	Homogeneous Groups
G 19833	38.000	A
G 2333	33.250	AB
LIC-04-1-3	32.875	AB
PVA 773	32.250	AB
G 21212	29.875	ABC
AFR 708	29.375	ABCD
BAT 477	28.000	ABCDE
RAB 655	27.000	BCDEF
A 774	26.375	BCDEF
CAL 96	26.250	BCDEF
AFR 298	25.500	BCDEFG
CAL 143	25.375	BCDEFG
Carioca	25.125	BCDEFG
SER 16	25.000	BCDEFG
SXB 418	24.375	BCDEFG
SXB 412	23.750	BCDEFG
VAX 1	23.625	BCDEFG
BONUS	23.250	BCDEFG
DRK 16	23.250	BCDEFG
ICA PIJAO	22.875	BCDEFG
DOR 500	21.250	CDEFG
D.CALIMA	21.000	CDEFG
G 4523 (IP)	21.000	CDEFG
DOCTOR	20.875	CDEFG
T.CANELA	19.375	DEFG
SEA 15	18.000	EFG
DOR 364	17.625	EFG
SAB 258	17.375	FG
SEQ 1003	17.250	FG
SEA 5	15.625	G

Alpha 0.05, Standard Error for Comparison 2.7955, Critical Q Value 5.299. Critical Value for comparison 10.475. Error term used: Year*Rep*Genotype, 177 DF. There are 7 groups (A, B, etc.) in which the means are not significantly different from one another.

3) Basal root growth angle

Tukey HSD All-Pairwise Comparisons Test of Basal Root growth Angle for Genotype

Genotype	Mean	Homogeneous Groups
G 2333	6.2500	A
VAX 1	6.1250	AB
SER 16	5.8750	ABC
DOR 364	5.7500	ABCD
BAT 477	5.6250	ABCD
RAB 655	5.6250	ABCD
SEA 5	5.5000	ABCD
SEQ 1003	5.5000	ABCD
SAB 258	5.3750	ABCD
SEA 15	5.3750	ABCD
G 21212	5.2500	ABCDE
T.CANELA	5.2500	ABCDE
DOR 500	4.8750	ABCDEF
SXB 418	4.6250	ABCDEF
DRK 16	4.1250	ABCDEF
SXB 412	4.1250	ABCDEF
CAL 96	4.0000	ABCDEF
Carioca	3.8750	BCDEF
AFR 298	3.7500	CDEF
A 774	3.6250	CDEF
BONUS	3.6250	CDEF
ICA PIJAO	3.6250	CDEF
D.CALIMA	3.5000	DEF
DOCTOR	3.5000	DEF
G 4523 (IP)	3.5000	DEF
PVA 773	3.0000	EFG
LIC-04-1-3	3.0000	EFG
AFR 708	2.8750	FG
CAL 143	2.6250	FG
G 19833	1.1250	G

Alpha 0.05, Standard Error for Comparison 0.6182. Critical Q Value 5.299. Critical Value for comparison 2.3162. Error term used: Year*Rep*Genotype, 177 DF. There are 7 groups (A, B, etc.) in which the means are not significantly different from one another.

4) Basal root number

Tukey HSD All-Pairwise Comparisons Test of Basal Root Number for Genotype

Genotype	Mean	Homogeneous Groups
DOCTOR	11.500	A
LIC-04-1-3	11.500	A
AFR 298	11.000	AB
PVA 773	10.625	ABC
CAL 143	9.500	ABCD
D.CALIMA	9.375	ABCDE
AFR 708	9.375	ABCDE
DRK 16	9.250	BCDEF
CAL 96	9.125	BCDEFG
G 19833	8.750	CDEFGH
DOR 364	7.750	DEFGHI
BONUS	7.750	DEFGHI
G 4523 (IP)	7.625	DEFGHIJ
DOR 500	7.500	DEFGHIJ
SAB 258	7.375	DEFGHIJ
BAT 477	7.375	DEFGHIJ
Carioca	7.250	EFGHIJ
SEQ 1003	7.250	EFGHIJ
SER 16	7.250	EFGHIJ
SXB 418	7.250	EFGHIJ
G 2333	7.125	FGHIJK
G 21212	7.000	GHIJK
SEA 5	7.000	GHIJK
SXB 412	6.875	HIJK
T.CANELA	6.875	HIJK
VAX 1	6.750	HIJK
A 774	6.750	HIJK
ICA PIJAO	6.375	IJK
SEA 15	5.500	JK
RAB 655	5.000	K

Alpha 0.05 Standard Error for Comparison 0.5725. Critical Q Value 5.299. Critical Value for comparison 2.1453. Error term used: Year*Rep*Genotype, 177 DF. There are 11 groups (A, B, etc.) in which the means are not significantly different from one another.

5) Basal root whorl number

Tukey HSD All-Pairwise Comparisons Test of Basal Root Whorl Number for Genotype

Genotype	Mean	Homogeneous Groups
LIC-04-1-3	3.6250	A
DOCTOR	3.5000	AB
CAL 143	3.0000	BC
PVA 773	3.0000	BC
AFR 298	2.8750	C
AFR 708	2.7500	CD
DRK 16	2.7500	CD
D.CALIMA	2.7500	CD
CAL 96	2.5000	CDE
G 19833	2.5000	CDE
G 4523 (IP)	2.2500	DE
BONUS	2.1250	E
DOR 364	2.1250	E
A 774	2.0000	EF
BAT 477	2.0000	EF
Carioca	2.0000	EF
DOR 500	2.0000	EF
G 21212	2.0000	EF
G 2333	2.0000	EF
ICA PIJAO	2.0000	EF
SAB 258	2.0000	EF
SEA 5	2.0000	EF
SEQ 1003	2.0000	EF
SER 16	2.0000	EF
SXB 412	2.0000	EF
SXB 418	2.0000	EF
T.CANELA	2.0000	EF
VAX 1	2.0000	EF
RAB 655	1.5000	FG
SEA 15	1.3750	G

Alpha 0.05 Standard Error for Comparison 0.1423. Critical Q Value 5.299. Critical Value for comparison 0.5332. Error term used: Year*Rep*Genotype, 177 DF. There are 7 groups (A, B, etc.) in which the means are not significantly different from one another.

6) Primary root length

Tukey HSD All-Pairwise Comparisons Test of Primary Root Length for Genotype

Genotype	Mean	Homogeneous Groups
BAT 477	8.0000	A
AFR 298	7.5000	AB
DOR 364	7.5000	AB
RAB 655	7.5000	AB
SXB 412	7.5000	AB
T.CANELA	7.5000	AB
DOR 500	7.3750	AB
LIC-04-1-3	7.3750	AB
A 774	7.3750	AB
SEQ 1003	7.3750	AB
BONUS	7.2500	AB
D.CALIMA	7.1250	AB
CAL 143	7.1250	AB
Carioca	7.1250	AB
ICA PIJAO	7.1250	AB
SEA 15	7.1250	AB
AFR 708	7.0000	AB
G 2333	7.0000	AB
SAB 258	7.0000	AB
SXB 418	7.0000	AB
SEA 5	6.8750	AB
SER 16	6.8750	AB
CAL 96	6.7500	AB
G 21212	6.7500	AB
PVA 773	6.7500	AB
DRK 16	6.6250	AB
G 4523 (IP)	6.6250	AB
VAX 1	6.6250	AB
G 19833	6.6250	AB
DOCTOR	6.0000	B

Alpha 0.05 Standard Error for comparison 0.4884 Critical Q Value 5.299 Critical Value for comparison 1.8302. Error term used: Year*Rep*Genotype, 177 DF. There are 2 groups (A and B) in which the means are not significantly different from one another.

Chapter 3 Appendix

Appendix 3-1: Description of 155 genotypes from the bean core collection from CIAT evaluated in the field in Rock Springs.

Means of 4 replications of 14 traits are presented.. Adventitious root number (ARN), length in cm (ARL), branching (ARB) and diameter in mm (ARD); Basal root whorl number (BRWN); Basal root number (BRN), length in cm (BRL), branching (BRB), diameter in mm (BRD) and angle in degree (BRGA); Primary root length in cm (PRL), branching (PRB) and diameter (PRD) and number of nodules per plant, Shoot dry weight in grams per plant (SDW). Branching correspond to number of lateral roots in 2 cm root segment. ARN, BRWN and BRN are counts per plant. Gene pool: A - Andean and M, MI – Mesoamerican; Origin of the accessions: CLB – Colombia, GTA – Guatemala, BZL – Brazil, CRA – Costa Rica, MEX – Mexico, PER – Peru, HTI – Haiti, JMC – Jamaica, NCA – Nicaragua, ECD – Ecuador, ELS – El Salvador, CLE-Chile, CBA-Cuba, DOM-Dominican Republic, USA-United States of America. Bean races: NG1-Nueva Granada, group 1, NG2-Nueva Granada, group 2, P1-Peru, group 1, G-Guatemala, D1-Durango, group 1, D2-Durango, group 2, M1-Mesoamericana, group 1, M2-Mesoamericana, group 2. GH-growth habit: 1-determinado bush, 2-Indeterminado upright bush, 3-indeterminate semi-viney prostrate.

Code	Genotype	Plat	Race	Origin	GH	ARN	ARL	ARB	ARD	BRW	BRN	BRL	BRB	BRD	BRGA	PRL	PRB	PRD	Nodule	SDW
1	AND1005	A.	NG2	CLB	2	14.00	16.00	6.75	0.83	2.3	8.5	16.00	7.25	1.90	32.5	16.00	8.75	1.78	30.00	38.18
2	DRK47	A.	P1	CLB	1	17.50	16.50	6.00	1.20	2.8	10.5	22.75	6.00	2.90	33.8	17.25	8.25	2.68	39.00	33.43
3	G738	A.	NG1	GTA	1	15.50	12.25	3.75	0.85	3.0	11.3	25.75	5.75	2.48	50.0	19.00	6.75	2.28	29.00	31.28
5	G1678	A.	NG2	BZL	2	13.75	14.75	5.50	0.48	2.0	7.8	21.75	6.25	1.53	53.8	23.50	6.25	2.25	13.75	36.12
6	G1688	A.	NG1	BZL	2	16.50	7.00	5.50	0.40	2.3	7.8	15.25	5.75	1.88	36.3	13.50	7.25	2.60	9.50	34.42
7	G1836	A.	NG1	CRA	2	12.00	9.25	8.50	0.65	2.5	8.5	19.75	9.25	1.70	35.0	14.50	7.50	1.95	26.00	35.86
8	G1939	A.	NG1	MEX	2	14.50	14.50	6.50	0.78	1.8	6.5	18.75	5.50	2.15	33.8	12.75	6.75	2.40	23.25	40.52
10	G2567	A.	P1	ECD	2	15.50	9.50	6.25	0.70	2.8	10.0	18.50	7.25	1.75	42.5	19.25	7.75	2.73	42.50	35.99
11	G2686	A.	P1	PER	1	21.50	10.50	5.50	0.58	2.0	7.8	23.75	8.00	2.88	30.0	24.50	9.50	3.90	22.25	37.56
12	G2875	A.	NG1	MEX	2	11.75	10.50	6.00	0.55	2.5	9.3	18.50	8.00	2.00	52.5	12.50	9.25	2.05	9.00	47.78
13	G3157	A.	NG1	GTA	1	14.50	17.25	6.75	0.98	3.0	10.5	27.75	6.25	2.68	33.8	14.00	6.75	2.05	17.00	48.43
14	G4001	A.	NG1	CRA	2	12.75	11.00	5.75	0.70	2.8	10.5	20.00	6.00	2.83	25.0	16.75	6.00	2.60	46.50	41.04
15	G4534	A.	NG1	PER	1	6.75	13.50	5.50	0.58	2.8	8.5	24.00	7.75	2.43	22.5	17.50	6.75	2.80	17.25	39.03
16	G4547	A.	P1	CLB	1	13.75	19.75	7.75	0.88	2.3	8.8	26.75	7.25	2.53	56.3	20.50	7.50	2.45	65.50	31.77
17	G4644	A.	NG2	CLB	1	6.50	18.00	5.25	0.80	2.5	8.3	22.00	6.25	2.20	48.8	20.00	8.50	2.35	27.50	42.19
18	G4721	A.	P1	PER	2	16.75	11.50	5.00	0.48	3.0	10.8	19.50	5.50	2.28	25.0	17.50	6.50	2.75	24.25	37.33
19	G4739	A.	P1	PER	2	18.75	20.00	6.00	1.05	2.0	6.8	18.50	7.75	1.88	45.0	17.75	5.50	2.13	49.75	37.33
21	G5034	A.	NG2	BZL	1	13.00	19.00	6.75	0.55	2.0	7.8	27.00	6.50	2.05	46.3	17.75	7.25	2.28	20.25	33.92
22	G5142	A.	NG1	MEX	2	12.50	12.25	6.25	0.45	2.3	8.3	20.00	6.00	1.98	52.5	14.75	7.75	2.60	12.75	42.38
23	G5170	A.	NG2	BZL	1	11.00	18.00	5.75	0.98	2.3	8.5	23.75	7.75	2.45	32.5	15.00	6.00	2.20	28.00	32.78
24	G5273	A.	NG1	MEX	2	18.25	11.75	6.25	0.55	2.3	7.8	20.00	7.75	2.30	56.3	19.00	5.75	2.45	25.00	39.97
25	G5625	A.	NG1	MEX	1	9.50	14.25	7.00	0.70	3.0	11.8	23.00	4.75	2.65	27.5	15.75	7.75	2.90	19.50	51.91
26	G5708	A.	NG2	CLB	1	17.75	15.75	7.25	1.30	2.0	7.0	25.00	7.75	3.13	23.8	20.50	9.25	2.78	29.25	34.41
28	G5849	A.	NG2	CLE	3	12.50	11.75	3.50	0.35	2.5	9.5	15.00	5.25	1.48	51.3	10.00	5.25	2.13	14.00	38.09
30	G6639	A.	NG1	HTI	1	17.25	13.00	7.75	0.95	2.3	8.0	23.75	6.00	2.18	38.8	20.50	6.25	2.65	60.75	29.38
31	G6873	A.	NG2	BZL	1	17.25	5.75	4.50	0.48	2.8	9.8	23.25	9.25	1.88	30.0	13.75	8.00	2.30	10.00	29.66

Code	Genotype	Plat	Race	Origin	GH	ARN	ARL	ARB	ARD	BRW	BRN	BRL	BRB	BRD	BRGA	PRL	PRB	PRD	Nodule	SDW
32	G7776	A.	NG1	ECD	2	21.00	12.50	6.75	0.48	2.8	9.5	20.00	7.00	2.15	40.0	15.25	8.00	1.28	60.00	37.00
33	G7895	A.	NG2	PER	1	13.25	13.75	9.75	0.88	2.5	8.0	24.25	8.50	2.73	30.0	18.75	10.00	2.98	56.50	40.58
34	G7945	A.	NG1	HTI	1	10.00	12.00	6.00	0.53	3.0	11.0	29.75	6.25	2.65	17.5	15.00	7.50	2.83	11.00	39.21
35	G8209	A.	P1	PER	3	19.00	13.00	6.50	0.55	2.3	8.0	21.00	7.50	2.28	48.8	16.00	8.50	2.48	46.75	46.87
36	G9603	A.	NG2	BZL	3	25.25	7.50	7.75	0.38	2.3	7.5	17.50	11.25	1.65	38.8	13.50	7.00	2.33	22.25	33.56
37	G9846	A.	NG1	ECD	1	16.00	13.50	6.25	0.78	2.3	7.5	23.50	6.50	2.50	40.0	23.00	6.50	2.50	53.25	45.18
38	G11512	A.	NG2	ECD	1	15.50	20.50	6.75	0.65	2.5	8.5	23.00	7.25	2.80	36.3	16.25	7.75	1.85	36.75	35.42
39	G11521	A.	P1	ECD	1	21.75	18.00	7.00	1.35	2.8	9.8	22.50	6.50	2.15	41.3	18.00	10.50	2.68	45.25	35.51
40	G11564	A.	NG2	ECD	2	16.75	16.75	6.50	1.40	2.8	9.8	24.00	8.25	2.23	41.3	14.25	7.75	1.73	59.75	35.33
41	G11585	A.	NG2	PER	1	17.00	15.75	6.75	0.90	1.8	7.0	24.00	8.50	2.53	17.5	14.25	9.75	2.50	96.75	40.49
42	G11723	A.	NG2	PER	2	18.50	20.25	7.50	0.50	2.3	8.8	21.50	7.50	2.60	40.0	19.75	7.50	2.33	42.25	34.50
43	G11727	A.	NG2	PER	3	21.25	14.00	6.00	0.65	2.5	9.0	21.00	6.25	2.38	32.5	18.25	7.25	3.05	28.75	37.37
45	G11759A	A.	NG2	PER	2	20.75	16.00	5.50	0.78	2.3	8.0	17.25	7.00	1.60	53.8	15.25	7.75	1.63	21.75	30.36
47	G11957	A.	NG1	MEX	3	17.75	17.00	4.75	0.53	2.0	7.0	18.50	6.50	1.93	55.0	17.75	7.00	2.08	11.75	33.64
53	G12689	A.	NG1	CLB	1	10.25	9.25	5.50	0.38	2.5	9.0	22.25	7.00	1.83	38.8	12.25	7.00	2.63	27.25	30.78
54	G13094	A.	NG1	MEX	1	7.75	13.75	6.50	0.40	2.8	10.0	19.75	7.50	2.03	26.3	21.75	8.75	3.15	31.25	34.02
55	G14253	A.	NG2	PER	1	25.25	17.50	6.50	0.38	2.3	8.8	22.25	7.25	1.73	22.5	14.00	9.25	2.43	11.50	33.67
56	G14659	A.	P1	ECD	2	21.25	18.00	6.00	0.48	2.8	10.0	20.50	7.75	1.98	31.3	15.00	6.25	2.68	20.75	34.03
58	G16104E	A.	NG2	PER	1	12.00	15.25	5.00	0.63	2.8	9.3	21.25	6.75	1.95	32.5	13.75	10.50	2.23	37.25	41.38
59	G16115	A.	NG1	PER	1	26.50	17.25	7.00	0.75	2.5	8.8	25.25	4.25	2.80	40.0	27.50	9.50	3.20	29.25	37.03
60	G17070	A.	NG1	ECD	1	18.50	15.25	7.00	0.73	3.0	10.0	24.25	5.50	3.30	32.5	18.50	6.00	2.93	22.75	45.89
61	G17076	A.	NG1	ECD	1	11.00	14.50	6.25	0.55	2.3	9.0	21.50	6.25	2.55	26.3	20.25	5.75	3.33	40.50	40.61
62	G17168	A.	NG2	ECD	2	21.50	5.50	6.00	0.63	2.3	7.8	25.00	8.75	2.65	56.3	18.75	6.00	3.03	37.25	32.48
63	G18148	A.	NG1	HTI	1	14.25	4.50	2.25	0.20	2.0	7.5	21.50	6.00	1.23	46.3	15.00	5.75	1.90	3.00	28.97
64	G18255	A.	NG1	CBA	1	16.25	19.50	8.25	0.93	3.0	9.3	20.25	6.00	2.35	36.3	21.25	6.50	2.75	24.50	47.82
65	G18264	A.	NG2	DOM	3	18.25	14.50	8.50	0.73	2.3	8.0	28.00	7.00	2.15	52.5	21.25	11.75	2.93	34.50	33.34

Code	Genotype	Plat	Race	Origin	GH	ARN	ARL	ARB	ARD	BRW	BRN	BRL	BRB	BRD	BRGA	PRL	PRB	PRD	Nodule	SDW
66	G18942	A.	NG1	BZL	1	4.50	6.00	5.25	0.58	2.5	8.0	22.50	5.25	2.43	40.0	16.25	5.00	2.53	17.25	48.12
68	G19833	A.	P1	PER	3	28.00	19.75	8.00	0.68	2.8	10.3	19.25	7.00	1.75	25.0	13.00	8.00	2.00	94.50	35.66
70	G19848	A.	NG2	PER	3	10.00	14.00	6.25	1.45	3.0	10.3	21.00	7.00	2.50	35.0	15.25	7.25	2.15	62.50	43.60
84	DOR364	M.	NG2	CLB	2	14.50	11.50	3.50	0.23	2.0	7.8	25.25	5.25	2.65	47.5	19.00	5.75	4.03	8.25	42.34
85	G278	M.	M1	ELS	3	28.75	12.50	6.00	0.38	2.0	7.5	15.75	5.25	1.35	63.8	16.00	5.00	2.03	18.50	35.01
86	G753	M.	D2	MEX	3	17.50	5.75	4.00	0.33	1.8	6.8	24.50	5.75	2.15	30.0	18.50	5.25	2.35	4.00	48.09
87	G801	M.	D2	GTA	3	38.50	17.00	7.00	0.58	1.8	7.8	22.50	5.50	1.93	56.3	16.25	6.00	2.63	34.50	43.82
88	G803	M.	M2	NCA	3	24.50	15.00	5.75	0.35	2.0	7.8	22.00	6.25	1.75	70.0	17.50	6.25	2.75	26.50	39.39
89	G955	M.	M2	ELS	3	18.25	16.00	6.00	0.48	2.3	7.0	18.00	5.50	1.98	42.5	13.75	7.25	3.03	15.25	42.14
90	G1328	M.	M1	CRA	3	14.25	15.75	5.75	0.68	1.0	4.0	29.50	7.50	2.30	57.5	18.75	6.25	2.80	47.00	34.96
91	G1797	M.	G	MEX	3	21.25	12.75	6.00	0.68	2.3	8.3	21.50	7.25	1.33	65.0	19.25	8.00	1.98	32.00	36.76
92	G1957	M.	D1	MEX	3	35.00	16.25	7.75	0.43	2.0	7.3	20.50	5.25	2.15	22.5	16.25	6.25	2.50	36.50	31.13
93	G2093	M.	M2	GTA	2	18.25	14.50	4.25	0.80	2.0	7.3	20.75	6.00	2.23	50.0	16.25	6.00	2.73	42.50	41.27
94	G2137	M.	M1	NCA	3	28.00	10.00	9.25	0.45	2.0	7.3	22.00	7.50	1.33	56.3	11.25	6.00	2.13	41.50	35.87
95	G2277	M.	M1	NCA	3	31.75	22.25	5.25	1.10	2.0	7.8	24.00	6.00	2.08	48.8	13.50	4.75	2.23	31.25	44.48
96	G2348	M.	G	MEX	3	24.50	17.50	4.25	0.43	2.0	7.0	28.00	6.00	2.20	38.8	14.25	5.50	2.65	7.50	32.37
97	G2352	M.	M1	MEX	3	18.25	9.50	3.75	0.35	1.5	6.3	20.75	5.75	2.45	27.5	17.00	6.50	3.30	17.50	45.36
98	G2402	M.	M1	MEX	3	17.75	19.50	7.00	0.38	2.0	7.5	19.00	5.50	1.90	73.8	15.75	7.50	3.63	13.00	35.74
99	G2445	M.	D1	MEX	3	18.00	9.50	5.00	0.30	2.0	7.8	20.75	7.25	2.00	42.5	17.50	8.50	2.73	33.00	35.00
100	G2635	M.	M1	MEX	3	36.75	11.50	8.75	0.50	1.0	4.0	23.50	6.00	1.80	60.0	12.75	6.50	2.85	14.25	39.96
101	G2660	M.	D1	MEX	3	40.75	14.25	5.00	0.38	2.0	7.0	22.25	8.25	1.75	72.5	13.50	7.00	2.15	49.75	39.50
102	G2775	M.	G	MEX	3	34.25	17.75	4.00	0.33	2.3	8.0	22.75	5.50	2.25	56.3	13.50	6.00	2.08	17.25	48.19
103	G2778	M.	D1	MEX	3	22.75	17.75	5.25	0.48	1.5	5.0	20.75	6.50	1.60	66.3	15.00	4.25	2.40	25.50	27.43
104	G2866	M.	D1	MEX	3	17.00	15.25	6.75	0.58	2.0	8.3	20.50	7.50	1.75	55.0	15.50	8.75	2.23	9.25	40.96
105	G2997	M.	D1	MEX	3	27.00	13.50	5.50	0.45	2.3	8.3	21.00	5.50	1.63	47.5	17.00	6.00	2.88	10.50	29.83
106	G3005	M.	M1	GTA	2	19.50	9.00	3.75	0.40	2.0	8.0	17.00	5.00	1.78	30.0	18.25	9.00	3.18	12.00	35.72

Code	Genotype	Plat	Race	Origin	GH	ARN	ARL	ARB	ARD	BRW	BRN	BRL	BRB	BRD	BRGA	PRL	PRB	PRD	Nodule	SDW
107	G3017	M.	M2	GTA	3	21.00	12.50	4.75	0.20	2.5	9.5	24.50	6.00	1.88	56.3	17.75	6.50	2.80	23.25	34.20
108	G3142	M.	M2	GTA	3	16.25	9.75	3.75	0.40	2.0	7.5	23.25	5.75	2.50	55.0	15.50	7.75	2.33	12.00	51.86
109	G3178	M.	M2	GTA	3	30.25	21.00	6.50	0.88	2.0	7.5	20.25	6.25	1.55	50.0	9.25	8.25	2.15	24.00	47.94
110	G3185	M.	M2	GTA	3	28.50	7.75	4.00	0.60	2.0	7.5	24.25	7.75	2.15	17.5	17.50	7.25	3.33	19.00	47.63
111	G3217	M.	M2	GTA	2	19.00	17.25	4.25	0.75	2.0	7.5	25.25	6.50	2.13	51.3	17.00	9.00	3.00	8.25	29.20
112	G3545	M.	M2	GTA	2	28.00	10.50	4.50	0.28	2.0	7.8	26.25	7.00	2.25	48.8	15.75	7.00	2.83	13.75	36.32
113	G3586	M.	M1	MEX	3	15.00	6.50	1.50	0.28	1.8	6.8	20.00	5.00	2.63	36.3	16.75	6.00	3.33	1.50	35.91
114	G3593	M.	M2	MEX	2	15.00	7.50	4.75	0.18	2.0	7.5	18.75	5.75	2.40	26.3	15.00	7.75	3.05	9.00	36.66
115	G3595	M.	M1	MEX	3	21.25	11.25	4.50	0.38	2.0	7.5	20.50	5.75	1.85	43.8	17.75	6.00	3.13	9.75	28.91
116	G3661	M.	M1	CLB	3	37.00	13.00	4.50	0.33	2.0	8.0	23.00	8.25	1.95	45.0	21.00	7.75	2.80	23.50	39.92
117	G3807	M.	M1	ELS	1	16.00	23.25	8.25	0.98	2.3	8.8	23.75	6.00	2.10	42.5	16.25	8.00	2.80	27.25	35.91
118	G3936	M.	D2	BZL	3	26.50	10.75	4.00	0.48	2.0	6.8	28.25	6.75	1.98	56.3	18.50	7.00	2.73	46.00	36.26
128	G4822	M.	G	PER	2	15.50	10.50	7.25	0.28	2.0	7.5	19.75	8.00	2.08	40.0	19.25	11.25	3.55	29.25	45.96
129	G5036	M.	D2	BZL	2	20.50	12.50	5.00	0.23	2.0	7.5	24.25	6.00	2.53	22.5	19.50	4.75	3.00	10.75	30.38
130	G5653	M.	M1	BZL	3	50.00	9.50	7.75	0.40	1.3	5.0	27.00	7.25	2.55	75.0	17.75	6.75	2.40	7.50	30.98
131	G5694	M.	G	ECD	2	17.00	10.50	5.25	0.48	2.0	8.3	23.50	5.25	2.60	36.3	14.00	8.00	3.93	11.25	40.80
132	G5712	M.	M1	USA	3	18.75	13.25	5.00	0.58	2.0	8.5	25.00	6.75	1.98	58.8	15.75	7.75	1.95	32.50	31.87
133	G5733	M.	M2	GTA	3	13.50	7.50	4.25	0.30	2.0	7.5	29.25	6.25	2.40	51.3	18.25	5.75	3.33	42.50	40.23
134	G6450	M.	M2	JMC	2	15.75	19.50	6.00	1.00	3.0	10.8	20.75	7.75	2.28	53.8	15.25	8.25	3.13	30.25	46.60
135	G7038	M.	M1	ECD	3	21.25	11.50	5.25	1.28	2.0	7.5	21.50	5.75	2.38	56.3	11.25	6.25	3.00	25.00	43.98
137	G7742	M.	D1	MEX	3	8.50	17.00	6.75	0.58	1.5	5.8	19.25	5.25	1.53	60.0	19.00	6.75	2.28	26.50	40.78
138	G7761	M.	D2	MEX	3	25.00	9.75	7.00	0.53	2.0	7.3	21.50	5.25	2.08	47.5	16.25	7.00	2.58	37.75	27.13
139	G7765	M.	M2	MEX	2	25.50	8.00	5.50	0.33	2.0	7.5	21.75	7.50	2.40	43.8	19.00	6.75	2.90	21.00	31.13
140	G7863	M.	M2	CLB	3	12.50	7.00	4.50	0.23	2.0	7.0	23.00	4.50	2.18	45.0	14.25	5.75	2.98	20.25	30.52
148	G14914	M.	G	MEX	3	19.25	11.25	4.25	0.23	2.0	7.0	19.25	6.00	1.93	15.0	13.00	7.50	2.05	42.75	30.87
150	G17648	M.	M1	BZL	3	9.50	12.75	3.50	0.28	1.8	6.8	25.50	4.75	1.85	33.8	19.00	5.00	2.80	9.25	31.89

Code	Genotype	Plat	Race	Origin	GH	ARN	ARL	ARB	ARD	BRW	BRN	BRL	BRB	BRD	BRGA	PRL	PRB	PRD	Nodule	SDW
151	G17649	M.	M1	GTA	3	19.00	20.50	5.50	0.38	2.0	8.0	25.25	6.75	2.28	55.0	20.00	6.50	2.03	23.25	22.37
152	G18440	M.	M2	GTA	3	15.00	17.75	5.75	0.53	2.0	8.0	25.00	6.50	1.80	46.3	14.50	9.50	2.73	9.50	32.03
153	G18446	M.	D1	MEX	3	30.00	16.25	4.25	0.30	2.0	7.3	20.25	6.50	1.93	62.5	16.75	5.50	1.75	22.25	27.27
154	G10945	M.	D1	MEX	3	19.75	13.00	4.25	0.30	2.0	7.5	21.00	6.50	2.18	53.8	17.50	7.25	2.20	19.50	40.84
155	G22044	M.	D1	MEX	3	32.25	13.25	5.50	0.38	1.3	5.0	16.75	6.00	1.93	60.0	18.25	9.25	2.58	8.00	31.47
156	BAT93	MI	D2	MEX	3	15.25	17.50	7.25	0.60	2.0	7.3	27.75	6.00	2.10	55.0	17.75	8.25	3.53	13.00	40.94
157	BAT477	MI	M2	CLB	3	23.00	13.75	6.50	0.30	2.0	8.3	18.75	7.50	1.75	43.8	15.25	7.25	2.50	11.50	53.77
158	G1264	MI	M1	MEX	2	20.00	10.50	3.00	0.23	2.0	7.5	25.50	7.00	2.20	40.0	19.00	6.00	4.50	12.50	41.49
159	G1356	MI	M2	MEX	2	17.50	14.00	4.25	0.48	2.0	7.8	22.50	6.00	1.68	42.5	16.25	6.50	2.45	6.75	26.59
160	G1358	MI	G	MEX	3	25.50	17.00	6.25	0.83	2.0	7.3	23.00	8.50	2.28	52.5	20.25	7.25	2.65	25.50	30.51
161	G1977	MI	M2	MEX	3	24.00	18.25	6.00	0.68	2.0	8.0	25.50	7.25	2.10	55.0	19.50	7.25	2.50	45.75	35.12
162	G2199	MI	M2	GTA	3	18.25	15.00	5.50	0.33	2.5	9.3	25.75	5.50	2.20	60.0	16.25	6.75	3.70	23.00	33.31
163	G2379	MI	M1	GTA	3	36.75	15.75	4.75	0.50	2.0	7.3	24.25	5.50	1.95	52.5	14.00	6.75	2.43	16.50	34.14
164	G3331	MI	D1	MEX	3	20.75	10.75	5.00	0.33	1.5	5.5	22.00	5.75	1.78	45.0	13.75	7.50	2.40	18.25	29.12
165	G3334	MI	D2	MEX	3	15.25	20.00	6.75	0.68	2.3	8.0	21.00	7.00	2.08	48.8	13.75	5.75	2.75	24.25	35.64
166	G3642	MI	D2	MEX	3	16.25	15.50	5.50	0.63	2.3	8.3	16.75	5.25	2.05	45.0	16.00	5.75	1.80	29.75	36.18
167	G4258	MI	NG2	BZL	3	10.25	15.00	7.75	0.83	2.8	9.3	24.25	6.00	2.48	57.5	22.25	7.75	3.15	22.75	38.39
168	G4278	MI	M2	GTA	3	40.50	12.75	10.25	0.35	2.0	7.0	22.00	8.25	2.10	52.5	14.00	7.75	2.60	42.50	42.13
169	G4494	MI	D2	MEX	1	16.50	18.25	7.25	1.03	2.8	10.3	25.25	5.00	2.23	35.0	16.50	7.00	2.85	12.25	34.73
170	G4672	MI	P1	CLB	3	35.25	16.00	7.75	0.75	2.0	7.8	24.50	8.75	1.58	57.5	14.25	8.00	1.55	78.00	39.33
172	G7952	MI	NG2	BZL	3	21.25	15.75	7.50	0.85	2.3	8.5	21.25	5.00	1.95	48.5	13.25	8.00	2.63	8.75	23.99
173	G9335	MI	M2	MEX	3	19.25	14.75	5.75	0.50	2.3	8.3	21.50	5.50	1.95	47.5	14.50	6.00	2.55	26.25	33.89
174	G9855	MI	NG2	BZL	2	18.00	11.25	4.75	0.48	2.5	9.3	28.00	6.25	1.95	27.5	16.75	6.00	2.60	28.25	29.21
175	G11057	MI	NG2	ECD	3	28.00	18.25	7.50	0.73	1.3	5.0	22.75	7.00	1.78	66.3	18.50	9.00	2.28	24.75	28.84
176	G11059	MI	D2	MEX	3	23.25	15.75	5.25	0.48	1.8	6.5	25.75	6.50	1.80	57.5	17.75	6.75	2.78	11.00	53.16
180	G12529	MI	P1	ECD	3	14.75	14.50	6.75	0.80	2.8	10.0	20.00	5.75	1.55	50.0	16.00	7.75	2.15	41.25	27.29

Code	Genotype	Plat	Race	Origin	GH	ARN	ARL	ARB	ARD	BRW	BRN	BRL	BRB	BRD	BRGA	PRL	PRB	PRD	Nodule	SDW
181	G12778	MI	P1	PER	3	40.50	7.00	5.00	0.43	2.3	8.0	25.50	8.50	1.73	57.5	14.25	7.50	2.78	37.25	35.07
182	G12796	MI	D2	MEX	3	23.25	11.50	6.00	0.18	1.8	6.3	22.75	5.50	1.83	63.8	15.00	8.50	3.15	32.00	38.88
183	G12806	MI	D2	MEX	2	23.75	13.75	6.25	0.48	1.8	7.3	24.75	5.50	2.33	36.3	18.75	6.75	4.20	14.00	41.43
184	G13177	MI	M2	MEX	3	24.50	12.75	5.25	0.48	2.0	7.3	26.00	5.50	1.85	62.5	19.00	5.25	2.75	18.25	46.67
185	G13578	MI	D1	MEX	2	17.25	10.50	4.00	0.50	2.3	8.3	25.25	6.00	2.80	43.8	16.75	7.50	3.83	11.50	50.22
186	G13595	MI	D2	BZL	3	16.50	17.00	6.00	0.68	2.3	8.8	23.50	6.00	1.18	42.5	16.00	7.50	1.60	16.25	39.90
187	G13696	MI	NG2	CBA	3	41.75	17.75	11.50	0.68	2.0	7.3	24.25	7.75	2.18	63.8	16.75	7.25	2.83	21.00	46.23
188	G13910	MI	D1	MEX	3	22.75	15.25	6.25	0.63	2.0	7.3	23.25	6.00	1.83	55.0	16.75	5.75	1.83	29.50	36.70
189	G13911	MI	NG2	ECD	3	31.00	17.00	6.00	0.90	2.3	8.3	24.75	6.75	2.10	60.0	20.25	6.75	2.08	20.00	35.29
190	G14016	MI	NG2	ECD	2	28.75	16.00	5.75	0.30	2.8	9.5	18.25	4.75	1.65	51.3	16.50	5.50	2.28	29.50	39.01
191	G14163	MI	P1	CLB	3	28.00	13.00	6.25	0.38	2.0	7.3	20.50	6.50	1.70	47.5	15.00	6.75	2.70	15.75	26.48
192	G15641	MI	M2	MEX	3	37.25	13.75	4.50	0.38	2.0	7.5	22.00	5.75	1.70	51.3	16.75	6.75	2.28	19.25	41.84
193	G15685	MI	M2	MEX	3	26.50	14.25	7.25	0.60	1.3	4.5	24.00	7.25	1.90	55.0	16.50	6.00	3.53	53.25	53.54
194	G15725	MI	D2	MEX	2	25.00	14.00	5.25	0.33	2.0	7.3	23.00	4.50	2.15	27.5	13.75	7.00	3.60	11.00	39.47
195	G16026	MI	M1	MEX	3	30.25	16.50	6.00	0.30	2.0	7.0	19.75	5.00	1.93	52.5	14.75	6.25	2.10	43.00	38.91
196	G16072	MI	D2	MEX	3	31.00	11.00	5.75	0.73	2.0	7.5	21.00	6.00	1.40	68.8	15.75	8.25	2.45	15.50	34.73
197	G16110A	MI	G	MEX	2	9.75	10.00	5.50	0.60	2.3	8.5	18.75	6.25	2.28	40.0	13.00	7.75	2.18	15.75	42.37
198	G16346	MI	NG2	PER	2	23.75	15.00	8.75	0.70	2.3	8.8	21.00	9.75	2.35	42.5	15.25	7.50	2.33	55.75	38.19
199	G16400	MI	NG2	ECD	3	27.50	15.25	7.00	0.38	2.0	7.5	22.00	6.50	2.90	40.0	17.00	7.00	3.65	31.50	35.49
200	G16401	MI	G	MEX	3	36.00	17.00	7.00	0.60	2.0	7.0	22.25	8.50	1.58	71.3	20.00	6.50	2.40	31.50	28.34
201	G16835	MI	G	MEX	3	14.50	11.00	4.75	0.43	1.8	6.3	26.75	6.50	2.63	61.3	17.50	6.25	3.63	14.75	28.60
202	G16849A	MI	M1	MEX	3	20.25	6.25	2.25	0.40	1.8	6.3	26.75	11.00	1.70	51.3	19.00	8.00	3.53	15.75	28.53
203	G18141	MI	M2	GTA	3	18.25	6.00	5.00	0.30	2.0	7.8	19.00	8.75	2.70	35.0	18.50	7.50	4.35	11.50	43.69
204	G18147	MI	M2	HTI	3	24.00	9.75	3.50	0.53	2.0	8.0	24.00	5.75	2.00	46.3	13.00	6.50	2.40	6.25	41.74
205	G18157	MI	M1	HTI	3	16.75	7.00	4.50	0.45	2.0	7.5	21.00	6.75	1.73	60.0	15.75	6.75	2.83	9.50	41.07

Appendix 3-2: Mean separation of root phenes with Tukey test by gene pool and by races evaluated in the field in Rock Springs, 2010.

Means with the same letter are not statistically different at 5% level of significance. Traits that ANOVA did not detected significant differences (Table 3 - 1) were not presented. The results are average of 155 bean accessions with 4 replications. Gene pool: A – Andean; B – Mesoamerican. Bean races: NG1 - Nueva Granada, group 1, NG2 - Nueva Granada, group 2, P1-Peru, group 1, G - Guatemala, D1 - Durango, group 1, D2-Durango, group 2, M1 - Mesoamericana, group 1, M2-Mesoamericana, group 2.

1. Adventitious root number (ARN)

Tukey HSD All-Pairwise Comparisons Test of ARN for Gene Pool

Gene Pool	Mean	Homogeneous Groups
M	23.352	A
A	14.758	B

Alpha 0.05, Standard Error for Comparison VARIES; Critical Q Value 2.772. Critical Value for Comparison VARIES; Error term used: Error, 594 DF. All 2 means are significantly different from one another.

Tukey HSD All-Pairwise Comparisons Test of ARN for Race

Race	Mean	Homogeneous Groups
P1	23.207	A
M1	19.707	AB
D1	19.642	AB
NG2	19.631	AB
D2	18.051	B
NG1	17.893	B
M2	17.224	B
G	17.085	B

Alpha 0.05; Standard Error for Comparison VARIES. Critical Q Value 4.285; Critical Value for Comparison VARIES. Error term used: Error, 594 DF. There are 2 groups (A and B) in which the means are not significantly different from one another.

2. Adventitious root branching (ARB)

Tukey HSD All-Pairwise Comparisons Test of ARB for GeneP

GeneP	Mean	Homogeneous Groups
M	6.2773	A
A	6.0391	A

Alpha 0.05 Standard Error for Comparison VARIES; Critical Q Value 2.772. Critical Value for Comparison VARIES; Error term used: Error, 594 DF. There are no significant pairwise differences among the means.

Tukey HSD All-Pairwise Comparisons Test of ARB for Race

Race	Mean	Homogeneous Groups
NG2	6.9498	A
P1	6.7637	A
NG1	6.5889	A
D2	6.3318	AB
D1	5.8770	AB
G	5.7864	AB
M2	5.6130	B
M1	5.3552	B

Alpha 0.05; Standard Error for Comparison VARIES. Critical Q Value 4.285; Critical Value for Comparison VARIES; Error term used: Error, 594 DF. There are 2 groups (A and B) in which the means are not significantly different from one another.

3. Adventitious root diameter (ARD)

Tukey HSD All-Pairwise Comparisons Test of ARD for Gene Pool

Gene Pool	Mean	Homogeneous Groups
A	0.7643	A
M	0.5447	B

Alpha 0.05; Standard Error for Comparison VARIES. Critical Q Value 2.772. Critical Value for Comparison VARIES Error term used: Error, 594 DF. All 2 means are significantly different from one another.

Tukey HSD All-Pairwise Comparisons Test of ARD for Race

Race	Mean	Homogeneous Groups
NG2	0.7567	A
P1	0.7484	A
D2	0.7208	AB
G	0.6627	AB
M1	0.6202	AB
D1	0.6138	AB
M2	0.5652	B
NG1	0.5484	B

Alpha 0.05; Standard Error for Comparison VARIES. Critical Q Value 4.285. Critical Value for Comparison VARIES. Error term used: Error, 594 DF. There are 2 groups (A and B) in which the means are not significantly different from one another.

4. Basal root whorl number (BRWN)

Tukey HSD All-Pairwise Comparisons Test of BRWN for Gene Pool

Gene Pool	Mean	Homogeneous Groups
A	2.4013	A
M	2.1890	B

Alpha 0.05; Standard Error for Comparison VARIES. Critical Q Value 2.772; Critical Value for Comparison VARIES; Error term used: Error, 594 DF. All 2 means are significantly different from one another.

Tukey HSD All-Pairwise Comparisons Test of BRWN for Race

Race	Mean	Homogeneous Groups
NG1	2.5497	A
P1	2.5149	AB
NG2	2.3768	AB
G	2.3078	ABC
D2	2.2737	BC
M2	2.1856	CD
D1	2.1422	CD
M1	2.0106	D

Alpha 0.05; Standard Error for Comparison VARIES. Critical Q Value 4.285; Critical Value for Comparison VARIES; Error term used: Error, 594 DF. There are 4 groups (A, B, etc.) in which the means are not significantly different from one another.

5. Basal root number (BRN)

Tukey HSD All-Pairwise Comparisons Test of BRN for Gene Pool

Gene Pool	Mean	Homogeneous Groups
A	8.7022	A
M	8.1263	B

Alpha 0.05; Standard Error for Comparison VARIES. Critical Q Value 2.772. Critical Value for Comparison VARIES. Error term used: Error, 594 DF. All 2 means are significantly different from one another.

Tukey HSD All-Pairwise Comparisons Test of BRN for Race

Race	Mean	D1	D2	G	M1	M2	NG1	NG2
D1	7.831							
D2	8.397	0.565						
G	8.367	0.536	0.029					
M1	7.538	0.293	0.858*	0.829				
M2	7.976	0.144	0.420	0.391	0.437			
NG1	9.135	1.303*	0.737	0.767	1.596*	1.158*		
NG2	8.739	0.907*	0.342	0.371	1.200*	0.763*	0.395	
P1	9.328	1.496*	0.931*	0.960	1.789*	1.352*	0.1933	0.588

Alpha 0.05; Standard Error for Comparison VARIES. Critical Q Value 4.285. Critical Value for Comparison VARIES. Error term used: Error, 594 DF. The homogeneous group format can't be used.

6. Basal root branching (BRB)

Tukey HSD All-Pairwise Comparisons Test of BRB for Gene Pool

Gene Pool	Mean	Homogeneous Groups
A	6.9629	A
M	6.3413	B

Alpha 0.05. Standard Error for Comparison VARIES. Critical Q Value 2.772. Critical Value for Comparison VARIES. Error term used: Error, 594 DF. All 2 means are significantly different from one another.

Tukey HSD All-Pairwise Comparisons Test of BRB for Race

Race	Mean	D1	D2	G	M1	M2	NG1	NG2
D1	6.6170							
D2	6.0615	0.5555						
G	7.0688	0.4518	1.0073					
M1	6.6554	0.0384	0.5940	0.4134				
M2	6.7333	0.1164	0.6719	0.3354	0.0779			
NG1	5.9635	0.6534	0.0979	1.1052	0.6919	0.7698		
NG2	7.1330	0.5160	1.0715*	0.0642	0.4776	0.3996	1.1694*	
P1	6.9846	0.3676	0.9232	0.0842	0.3292	0.2513	1.0211*	0.1484

Alpha 0.05. Standard Error for Comparison VARIES. Critical Q Value 4.285. Critical Value for Comparison VARIES. Error term used: Error, 594 DF. The homogeneous group format can't be used because of the pattern of significant differences.

7. Basal root diameter (BRD)

Tukey HSD All-Pairwise Comparisons Test of BRD for Gene Pool

Gene Pool	Mean	Homogeneous Groups
A	2.1588	A
M	1.9492	B

Alpha 0.05; Standard Error for Comparison VARIES. Critical Q Value 2.772. Critical Value for Comparison VARIES. Error term used: Error, 594 DF. All 2 means are significantly different from one another.

Tukey HSD All-Pairwise Comparisons Test of BRD for Race

Race	Mean	Homogeneous Groups
G	2.1672	A
M1	2.1166	A
NG1	2.1093	A
M2	2.1078	A
D2	2.0858	A
NG2	2.0379	A
D1	2.0104	A
P1	1.7971	A

Alpha 0.05; Standard Error for Comparison VARIES. Critical Q Value 4.285. Critical Value for Comparison VARIES. Error term used: Error, 594 DF. There are no significant pairwise differences among the means.

8. Basal root growth angle (BRGA)

Tukey HSD All-Pairwise Comparisons Test of BRGA for Gene Pool

Gene Pool	Mean	Homogeneous Groups
M	52.271	A
A	40.476	B

Alpha 0.05. Standard Error for Comparison VARIES. Critical Q Value 2.772. Critical Value for Comparison VARIES. Error term used: Error, 594 DF. All 2 means are significantly different from one another.

Tukey HSD All-Pairwise Comparisons Test of BRGA for Race

Race	Mean	Homogeneous Groups
P1	51.830	A
NG2	49.662	A
D1	47.640	A
NG1	46.730	A
M1	45.891	A
G	43.741	A
M2	43.601	A
D2	41.895	A

Alpha 0.05. Standard Error for Comparison VARIES. Critical Q Value 4.285. Critical Value for Comparison VARIES. Error term used: Error, 594 DF. There are no significant pairwise differences among the means.

9. Primary root branching (PRB)

Tukey HSD All-Pairwise Comparisons Test of PRB for Gene Pool

Gene Pool	Mean	Homogeneous Groups
A	7.6161	A
M	7.1462	B

Alpha 0.05. Standard Error for Comparison VARIES. Critical Q Value 2.772. Critical Value for Comparison VARIES. Error term used: Error, 594 DF. All 2 means are significantly different from one another.

Tukey HSD All-Pairwise Comparisons Test of PRB for Race

Race	Mean	Homogeneous Groups
G	7.8201	A
NG2	7.6615	A
D1	7.4595	A
P1	7.3804	A
D2	7.3508	A
M2	7.2499	A
M1	7.1274	A
NG1	6.9995	A

Alpha 0.05. Standard Error for Comparison VARIES. Critical Q Value 4.285. Critical Value for Comparison VARIES. Error term used: Error, 594 DF. There are no significant pairwise differences among the means.

10. Primary root diameter (PRD)

Tukey HSD All-Pairwise Comparisons Test of PRD for Gene Pool

Gene Pool	Mean	Homogeneous Groups
M	2.6323	A
A	2.3805	B

Alpha 0.05. Standard Error for Comparison VARIES. Critical Q Value 2.772. Critical Value for Comparison VARIES. Error term used: Error, 594 DF. All 2 means are significantly different from one another.

Tukey HSD All-Pairwise Comparisons Test of PRD for Race

Race	Mean	Homogeneous Groups
M1	2.7535	A
M2	2.7298	A
D2	2.6031	AB
NG1	2.5626	AB
G	2.4563	AB
P1	2.3762	AB
NG2	2.3502	B
D1	2.2196	B

Alpha 0.05. Standard Error for Comparison VARIES. Critical Q Value 4.285. Critical Value for Comparison VARIES. Error term used: Error, 594 DF. There are 2 groups (A and B) in which the means are not significantly different from one another.

11. Number of nodules (Nod)

Tukey HSD All-Pairwise Comparisons Test of Nnodule for Gene Pool

Gene Pool	Mean	Homogeneous Groups
A	32.129	A
M	26.740	B

Alpha 0.05. Standard Error for Comparison VARIES. Critical Q Value 2.772. Critical Value for Comparison VARIES. Error term used: Error, 594 DF. All 2 means are significantly different from one another.

Tukey HSD All-Pairwise Comparisons Test of Number of nodules for Race

Race	Mean	Homogeneous Groups
P1	38.826	A
D1	30.969	AB
NG2	30.633	AB
D2	29.593	AB
G	28.373	AB
M2	27.602	AB
M1	24.810	B
NG1	24.671	B

Alpha 0.05. Standard Error for Comparison VARIES. Critical Q Value 4.285. Critical Value for Comparison VARIES. Error term used: Error, 594 DF. There are 2 groups (A and B) in which the means are not significantly different from one another.

12. Score (1 to 9) for root rot infection

Tukey HSD All-Pairwise Comparisons Test of Root for Gene Pool

Gene Pool	Mean	Homogeneous Groups
A	1.5354	A
M	1.2254	B

Alpha 0.05. Standard Error for Comparison VARIES. Critical Q Value 2.772. Critical Value for Comparison VARIES. Error term used: Error, 594 DF. All 2 means are significantly different from one another.

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- PhD, Horticulture - Plant Breeding, 2013, Pennsylvania State University, University Park, PA, USA.
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RESEARCH EXPERIENCE

- Alternate host of pests and diseases of important crops in Moamba
- Virulence dynamics of bean rust pathogen and resistance to bean rust pathogen
- Bean breeding, pathology and physiology. Genetic basis of root hair traits in bean
- Genetic diversity of root traits in common bean, emphasis for low Phosphorus and drought tolerance adaptation
- Agro-ecological adaptability of rice and bean varieties in Mozambique

PRESENTATIONS

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- Statistics, Universidade Eduardo Mondlane, Mozambique, 1996-1997
- Plant pathology, Instituto Superior Politecnico de Gaza, Mozambique, 2008-2009
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