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THE ROLE OF ROOT GROWTH TRAITS IN RESISTANCE TO THE BIOTIC STRESS, FUSARIUM ROOT ROT AND THE ABIOTIC STRESS, LOW SOIL PHOSPHORUS IN COMMON BEAN (PHASEOLUS VULGARIS L.).

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KAREN ANN CICHY

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Karen Ann Cichy

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ABSTRACT

THE ROLE OF ROOT GROWTH TRAITS IN RESISTANCE TO THE BIOTIC STRESS, FUSARIUM ROOT ROT AND THE ABIOTIC STRESS, LOW SOIL PHOSPHORUS IN COMMON BEAN (PHASEOLUS VULGARIS L.).

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Genetic and environmental variability for root architecture has been identified in common bean. The overall objective of this research was to determine if root architecture traits are an adaptation against an abiotic and a biotic stress. The stresses compared in this study were the fungal disease Fusarium root rot caused by *Fusarium solani* f.sp. *phaseoli* (*Fsp*) and phosphorus deficient soils, both significantly reduce yield in major bean production regions.

Fusarium root rot is greatly influenced by environmental conditions that stress plant roots, which has hindered progress in breeding resistant bean cultivars. The first objective was to determine if Fusarium root rot resistance is conferred by genes expressed in the root or shoot and if root architecture plays a role in resistance. Reciprocal grafting of a resistant (FR266) and susceptible (Montcalm) bean cultivar was used to study resistance to *Fsp* in two root growth environments, one without additional stress (Experiment 1) and one with the additional stress of a compacted soil layer (Experiment 2). Reciprocal grafting revealed that root rot incidence was controlled by genes expressed only in the roots in Experiment 1. Variability for root architecture was present, but it did not affect root rot incidence. In Experiment 2, root and shoot genotype both dictated

root rot incidence and root traits including root length and root dry weight appeared to be related to root rot incidence.

Root architecture traits have been shown to be related to P uptake and influence tolerance to low P soils. The second objective was to study the relationship between root architecture and P uptake in an Andean recombinant inbred line (RIL) population developed from a cross between a low P tolerant (G19833) and susceptible (AND696) bean line. The RILs also varied in plant growth habit and this was examined in relation to root architecture and yield. The population was field-grown under both high and low P levels and root architecture traits, P uptake and seed yield were measured. A linkage map was developed and a QTL study was conducted to determine which regions of the genome controlled these traits. Two QTL for root length density were identified that explained nearly 40% of the phenotypic variation, but root traits were not important for low P tolerance as measured by P uptake and seed yield. Growth habit influenced yield differently across soil treatments and indeterminate RILs had higher root length density than determinate RILs in the high P treatment.

The third objective was to examine the relationship between tolerance to low P soil and seed P, Fe, and Zn levels and identify QTL controlling these traits in the population. Iron and Zn are important to human nutrition and interact with P stored as phytic acid. Variability for seed Fe and Zn levels related to seed P was detected and QTL for these traits co-localized to linkage group B01 near the region of the gene that controls determinate plant growth habit.

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Chapter 1: Literature Review: Breeding a Better Root System

Introduction:

To understand how root systems function as an organ, it is useful to classify roots based on type and distribution. Roots are often grouped into four broad classes, the taproot, basal roots, adventitious roots, and lateral roots (Zobel, 1991). The taproot or the primary root is of embryonic origin, basal roots originate from the root/shoot transition zone, adventitious roots originate from non-root tissue, and lateral roots originate from existing roots. Lateral roots can also be further classified into secondary and tertiary roots depending branching from the taproot (Fitter, 2002). There are some species specific specialized root types such as the proteiod root clusters, which are clusters of densely positioned lateral roots, found in the Proteaceae family. Additionally, some species have enlarged, starchy roots that are used as food crops, including carrot (*Daucus carota*), beet (*Beta vulgaris*), and sweet potato (*Ipomoea batatas*) (Austin, 2002).

Architecture describes the spatial configuration of the root system (Lynch, 1995). Root system architecture is formed by intrinsic developmental and response to environment type cues. Intrinsic mechanisms are those that are an essential part of plant development and allow for the characteristic architecture of a particular root system. Response mechanisms are pathways that determine how plants respond to external signals (Malamay 2005). The ability of a plant's root system to respond to particular environmental conditions to improve the survival in a specific environment is know as plasticity (Sultan, 1987).

Natural variation exists between and within plant species for intrinsic and response type mechanisms of root architecture development. For example, in a recombinant inbred line population from two ecotypes of Arabidopsis, two QTL were identified for lateral root length. One QTL acted intrinsically and the other was responsive to mannitol concentration (Gerald et al. 2006). Arabidopsis has been used successfully to identify root system architectural responses to nutrients N, P, and S as seen in Figure 1.

Both intrinsically and response regulated root system architectural traits can have a role in stress tolerance, and a growing number of studies measured genetic variation for root architecture and related that to stress avoidance or yield gains (Gerald et al., 2006; Laperche et al., 2006; Fita et al., 2006). A study of cultivated (*Lactuca sativa* L.) and wild (*L. serriola*) lettuce found that a QTL for taproot length co-localized with a QTL for moisture extracted from a deep soil zone (50-100 cm) (Johnson et al., 2000). Regions of the maize genome that explained genotypic variation for adventitious root mass co-localized with QTL for grain yield under water stress and non-water stress conditions, such that an increase in root mass was associated with an increase in yield at four separate QTL (Tuberosa et al., 2002). Adaptive plasticity for root mass and root length has also been observed in response to soil moisture, including drought and flooding stress in two *Polygonum* species (Bell and Sultan 1999).

The scope of this review is to examine the literature closely for attempts at breeding better root systems and relate where possible applications to common bean improvement. Common bean (*Phaseolus vulgaris* L.) is an excellent

candidate for improved root system architecture. It is an important grain legume often planted in marginal environments (Wortmann et al., 1998). Common bean is also very susceptible to a number of stresses.

Common bean roots have a large root length and root length density as compared to other annual legumes, soybean (*Glycine max* L.) and field pea (*Pisum sativum* L.). Common bean was found to have a smaller median root depth (0.46m) than soybean (0.56m) and field pea (0.48m) based on the average of three years of data on a Haplustoll soil (Merrill et al., 2002). Within *P.vulgaris*, genetic variability for root architecture traits exist and some of these traits have been implicated in stress resistance. For example, basal root shallowness is associated with bean genotypes tolerant to low phosphorus soils (Liao et al., 2001). The remainder of this literature review will focus on two stresses detrimental to bean production in many parts of the world, the abiotic stress, phosphorus deficiency, and the biotic stress, Fusarium root rot. The nature of each of these stresses and root system traits were examined along with other factors that may be useful for genetic control of these ailments.

Abiotic Stress: Phosphorus Deficiency

Phosphorus is an essential mineral for plant metabolic processes, including photosynthesis, synthesis and breakdown of carbohydrates, and energy transfer. Phosphorus is also a structural element of nucleic acids and phospholipids. Phosphorus levels of 0.3-0.5% plant dry weight are required for optimal plant growth (Marschner, 1995). Insufficient plant phosphorus levels result in reduced leaf size and number, flower set, and shoot to root ratios (Lynch et al., 1991).

Phosphorus is extremely reactive and exists in the soil as organic P or one of at least 170 different mineral phosphates. Organic P makes up 20 to 80% of the soil P, a large fraction of which is phytic acid (inositol hexaphosphate) (Richardson, 1994). Both mineral and organic P forms found in soils are insoluble, and therefore there are very low levels of P in the soil solution available for plant absorption at any one time (Holford, 1997). For most soils, the amount of P in solution is insufficient for crop production. There is an equilibrium that occurs with P in soil solution as plants absorb P (Whitney, 1988) (Figure 2). At the extreme end of low soil P availability are acid weathered soils, where P is in a complex with iron and aluminum. Such soils, specifically Andosols, Ultisoils, and Oxisols, make up 43% of the land area in the tropics. Many acid weathered soils adsorb or fix P so that 70 to 90% of P fertilizer applied will become part of these low solubility compounds (Sanchez and Salinas, 1981). Cultivation leads to mineralization of organic P and mineralization occurs rapidly in tropical soils as compared to temperate soils (Hedley et al., 1995).

Plants take up phosphorus from the soil solution in the form of orthophosphate (HPO_4^{-2} or $H_2PO_4^{-1}$). The relative amount of each anion depends on soil pH. Acid soils favor the formation of $H_2PO_4^{-1}$, and alkaline soils favor the formation of HPO_4^{-2}. Phosphorus uptake by plants roots across the cell boundary occurs against a steep concentration gradient via an ATP dependent proton symport (Bucher et al., 2001). Phosphorus uptake is greatest at the root tip, and is reduced by soil drying. Phosphorus diffusion is limited in the soil to less than 6 mm, therefore roots must be very close to phosphorus in the soil for uptake to

occur (Whitney, 1988). Root system surface area is very important for P uptake because the greater the surface area, the greater the potential contact with P anions (Lynch, 1995).

Phosphorus is translocated from the roots to young leaves in the xylem as inorganic P. Remobilization of P (in organic or inorganic form) from older leaves to younger leaves and roots occurs via the phloem and is more prevalent in P deficient plants (Schachtman et al., 1998).

Phosphorus deficient soils will continue to be a problem in the coming years, because P is a finite resource and the world's supply of rock phosphate is estimated to expire in 60-80 years (Beebe et al., 2006). Common bean is especially susceptible to low P soils in part due to the high P requirement associated with energy-intensive nodulation and N_2 fixation. For this reason, common bean genotypes tolerant to low P soils when N fertilizer is added may not be as tolerant if dependent on fixation to supply N to the plant (Christiansen and Graham, 2002). Low P soils are a major constraint to bean production in regions of Africa and Latin America where farmers lack access to sufficient P fertilizer (Wortmann et al., 1998).

Plant adaptation to low P soil:

Nutrient efficiency is the effectiveness with which a plant system utilizes nutrient inputs to produce outputs. Phosphorus efficiency, has been defined in numerous ways, here it is considered as the ability to produce plant growth and yield in relation to the amount of available phosphorus (Lynch and Beebe, 1995). Tolerance to low P soils, or phosphorus efficiency, can be achieved by two

distinct routes: acquisition efficiency and utilization efficiency. Acquisition efficiency reflects a plants ability to extract P from the environment. It has been shown to be related to root system traits that increase the root surface area and allow capture of more P form the soil, including specific root length, root hair density and mycorrhizae (Gahoonia and Nielsen, 2003). Root exudates that can free inorganic P from insoluble P forms are another form of acquisition efficiency (Marshcner, 1995).

Utilization efficiency is the superior ability of a plant to convert acquired P into plant biomass and yield. Possible P utilization efficiency mechanisms include reduced requirements and modified phenology. Increased seed P reserves is another form of utilization efficiency that may allow plants to get a better start in low P soils (Lynch and Beebe, 1995).

Phosphorus acquisition efficiency:

Root system traits that improve phosphorus acquisition efficiency, and act to capture more P from the soil by increasing the surface area of the root, have been studied extensively in bean and other crops. Complex root architectures may have evolved for the capture of immobile nutrients, such as P, from the soil. Even with greatly reduced lateral branching, Arabidopsis can capture sufficient nitrate ions (mobile), but require more lateral branching to effectively uptake sufficient phosphate ions (Fitter et al., 2002).

Genotypic variability for P acquisition efficiency has been identified in many crops including rice, soybean, and common bean (Wissuwa and Ae, 2001a; Yan et al., 1995ab; Li et al., 2005). Landraces of rice showed improved tolerance

to low P soils than improved varieties, suggesting there is still unexplored variation for P uptake that can be exploited for rice improvement (Wissuwa and Ae, 2001a). In common bean, wild germplasm was not more adapted to low P soils than cultivated genotypes (Araujo et al., 1998). A screening of thousands of both Mesoamerican landraces and Mesoamerican and Andean improved common bean varieties for tolerance to low fertility soil revealed variability within each of the groups, suggesting that selected genotypes of either type can be useful as parental material in a low fertility breeding program (Singh et al., 2003). According to Yan et al. (1995b), Andean common beans are superior to Mesoamerican beans in low P soils. They often have higher yields, although Mesoamerican lines are more responsive to P fertilization (Yan et al., 1995b). Phosphorus deficient soils of the tropics are of two major classes: mineral low P, including oxisols and utisols, with inorganic bound P, and volcanic low P soils, mostly andisols, with high organic matter and organic bound P. In a study of 16 common bean lines from the Andean and Mesoamerican gene pools, large genetic variation for P efficiency was discovered. The P acquisition efficiency appeared to be general and not soil specific (Yan et al., 1995a).

Root system architecture

Most studies that relate root architecture to P acquisition efficiency examine genotypes with diverse plant performance in P-limiting environments, eg. media with high and low levels of P and observe root responses. In maize, P-deficient media promotes larger root systems in P-efficient and P-inefficient genotypes. However, the P-efficient genotype had a larger root system and higher

total root length than the P-inefficient genotype in low P conditions (Lui et al., 2004). A plant growth simulation model for rice grown in low P showed that improving root growth was the best way to enhance tolerance to P deficiency (Wissuwa, 2003). Low P nutrient solution was associated with altered basal root growth angle of some common bean genotypes, a change not exhibited with deficiencies of N, K, Ca, Mg, S or micronutrients (Bonser et al., 1996). Rubio et al. (2003) found the shallowness of basal roots of common bean added a competitive advantage to shoot biomass production as compared to deep basal roots when P is not evenly distributed in soil depth but instead is found only in the topsoil. Phosphorus acquisition efficient bean genotypes have more lateral roots and shallower basal roots under low P conditions as compared to P inefficient genotypes grown under similar conditions (Lynch and Brown 2001). Low phosphorus insensitive Arabidopsis mutants did not switch from primary to lateral root growth as seen in wild type plants. The mutants also showed reduced expression of genes important in phosphate uptake, including a phosphate transporter and acid phosphatases (Sanchez-Calderon et al., 2006).

In a study of four common bean lines, efficient lines had more adventitious roots than inefficient lines. Differences were more pronounced early in development (22 days after planting) than later in development (43 days after planting) (Miller et al., 2003). This type of root system modification has implications for acquisition efficiency because adventitious roots are shallow and so are located within the P-enriched topsoil. Adventitious rooting can also be categorized as a P use efficiency mechanism because they tend to have a lower

carbon construction cost than other root types as they are less dense (Lynch and Ho, 2005).

The next step after the identification of root architecture traits associated with nutrient efficiency is to understand the genetic basis of these traits, and determine if the inheritance of a given trait is such that it can be readily introduced to inefficient germplasm.

Variability for tolerance to low P soils has been identified in the Andean gene pool (Yan et al., 1995a). In a population of recombinant inbred lines developed from a P acquisition efficient, large seeded Andean bean,G19833 and a P acquisition inefficient small seeded Mesoamerican bean DOR364, Liao et al. (2004) also found root gravitropism to be correlated with P use efficiency. These traits include basal root growth angle and shallow basal root length. Results from this growth pouch assay were correlated with field responses of bean to low P. Three QTL for root gravatropism traits all from G19833 detected using the growth pouch assay were associated with QTL for P uptake in low P field conditions. In the same population of RILs, a QTL for root length and one for specific root length on two different linkage groups co-localized with QTL for phosphorus uptake. The uptake QTL and the root QTL each explained about 15% of phenotypic variation for these traits (Beebe et al., 2006).

Root hairs

Root hairs, or trichoblasts are tubular outgrowths of rhizodermal cells. Since root hairs are thin, they are better able to explore soil for nutrients with low

mobility in soil such as P. They are able to penetrate cracks in the soil and also can penetrate moderately compacted soils. Root hairs therefore aid in plant anchorage. Root exudates are mainly released from root hairs. The growth of the root system and the movement of root hair zone allow the nutrient availability to be maintained spatially. (Jungk, 2001). Root hairs have many plasmodesmata between cells to aid in symplastic transport of nutrients to the vascular tissue. Root hair characteristics including length, radius, and density are important for P uptake (Bucher et al., 2001). Root hairs have been shown to be important for P use efficiency in bean, white clover, cowpea, wheat, and barley.

Yan et al. (2004) conducted a QTL mapping study of root traits associated with P use efficiency in 86 RILs of an Andean efficient (G19833) by Mesoamerica inefficient (DOR 364) cross in common bean. The efficient parent had higher root hair density, average root hair length and total root hair length. Root hair traits were induced by low P (2 μM) in both cultivars as compared to high P (1000 μM). H^+ exudation and total acid exudation increased in low P as compared to high P in both parents, and was higher in G19833 than DOR364.

Wang et al. (2004) analyzed root hair traits associated with P use efficiency in RILs of a wild x cultivated soybean cross. The wild genotype had a higher shoot biomass and P uptake. It also had higher root hair density and total root hair length per unit root length but lower average root hair length. None of these traits were however correlated with total P in the plant.

Mycorrhizae

Mycorrhizae are symbiotic associations between plant roots and fungi. They are widespread in nature. An estimated 83% of dicots and 79% of monocots have mycorrhizal associations (Marschner, 1995). Vesicular-arbuscular (VAM) is the only type of mycorrhizae associated with annual crop plants. In this symbiosis, host plants provide carbohydrates to the fungi and fungi primarily provide increased access to uptake of mineral nutrients to the host plant, especially for low mobility ions. Mycorrhizal plants take up 2-3 times more P per unit root length than non mycorrhizal plants (Tinker et al., 1992). The predominant mechanism by which VAM increase plant P uptake is by extending the surface area of the root system (Cardoso and Kuyper, 2006). In addition VAM can acquire inorganic P from some of the soil pools marginally available to plants, as shown in an experiment in an Oxisol soil with mycorrhizal and nonmycorrhizal maize (Cardoso et al., 2006). In the case of the phosphorus efficient bean cultivar 'Carioca', mycorrhizal plants were able to acquire more P from the soil than nonmycorrhizal plants because hyphae had a greater affinity for P in the soil solution than the plant roots and therefore a greater influx of P occurred. The beneficial effects of the mycorrhizal symbiosis on plant phosphorus levels were evident at flowering and mid podfill (deSilveira and Nogueira Cardoso, 2004).

Root exudates

Root exudates are able to increase phosphorus available to plants for uptake from the inorganic or organic pools not immediately available for plant

uptake (Figure 2). The type and amount of root exudate released by a plant is genotype dependent. Proteoid roots of white lupin, which are clusters of closely spaced determinate rootlets are very effective in acquiring P from the soil. They are known to release citrate and acid phosphatases which release P from organic and inorganic compounds (Gilbert et al., 1999). Lupin roots when compared to soybean roots, were able to accumulate 4.8 times more P per unit root length than soybean, suggesting that where soybean relied on increased root length to capture more P, lupin used root exudation to increase P availability (Watt and Evans, 2003). A study of exudates of three grain legumes, fava bean (*Vicia faba* L.), field pea (*Pisum sativum* L.) and white lupin (*Lupinus albus* L.) and the cereal spring wheat (*Triticum aestivum* L.) demonstrated all species' roots exude acid phosphatase, but the legumes more so than the wheat. There was also variability among species as to which soil P pools (inorganic and organic) were accessed (Nuruzzaman et al., 2006). A purple acid phosphatase gene isolated from *Medicago truncatula* was expressed in Arabidopsis roots and the transgenic plants were able to use organic P in the form of phytate as their sole P source (Xiao et al., 2006). This study illustrated the potential for P acquisition efficient crop plants to be developed via mechanisms that make plant unavailable P available. Another important point is that plant species and even genotypes have significant variability for the types and amounts of P solubilizing enzymes they contain and express.

In common bean, genotypic differences for root exudation of organic acids (citrate, acetate, and tartrate) also suggest a mechanism for P acquisition

efficiency. The large seeded Andean genotypes G19833 and G19839 exude more organic acids from their roots than Mesoamerican genotypes DOR364 and G212121. These organic acids are useful to mobilize Al and Fe bound P from soils for uptake by the plant roots (Shen et al., 2002). Low P was associated with an increase in acid phosphatase activity in the roots and on the root surface of the P-efficient genotype, whereas only slight increased acidity was found on the root surface of the inefficient genotype (Lui et al., 2004).

Root exudation is also soil type dependent. The cation-anion balance of the plant plays a role in the pH of the rhizosphere, which in turn can effect the P mobilization in the soil. The value of rhizosphere pH in mobilizing P is soil type dependent. In a luvisol, where P was mainly bound to Ca, a decrease in rhizosphere pH enhanced P mobilization, whereas in an oxisol where P was mainly bound to Al and Fe an increase in rhizosphere pH mobilized more P (Gahoonia and Nielsen 2004).

Phosphorus utilization efficiency:

An evaluation of 26 bean lines with varying levels of P efficiency, showed that the efficient and inefficient lines did not differ in P uptake, but differed in pods per plant and seeds per pod under low P conditions. At 65 days after emergence the efficient lines had a greater proportion of total P in pods and less in stems and leaves than that of the inefficient lines that had more P comparatively in stems and leaves and less in the pods (Youngdahl, 1990). A more in depth study of the P efficient common bean cultivar 'Calima' looked at remobilization of P from stem and leaves in low and high P growing media. The

study found that in low P media remobilization occurs earlier (56-70 days after emergence) than in high P media (70 days after emergence). Under low P conditions 80% of P stayed in the root system, whereas in high P only 20% stayed in the roots (Snapp and Lynch, 1996).

P use efficiency can also demonstrate itself in terms of carbon cost. A root system that requires less energy (i.e. lower root respiration) can increase P uptake per unit carbon invested. A comparison of P utilization in four common bean genotypes indicated that efficient and inefficient genotypes have a similar rate of P absorption per root dry weight. Phosphorus efficient bean genotypes G19839 and G1937 had a significantly lower rate of root respiration than the P inefficient genotypes DOR364 and Porillo Sintetico. The efficient genotypes had a higher percentage of their biomass as roots than the P-inefficient genotypes in low P soils (Nielsen et al., 2001).

A high level of seed P is considered to be a form of P use efficiency as it is indicative of the effect of P remobilization. In upland rice, the amount of shoot and root dry weight produced at six weeks of growth was highly correlated with the amount of phosphorus in the seed, suggesting high seed P as a trait to select for when breeding cultivars tolerant to low P soils (Hedley et al., 1994). A greenhouse experiment with four annual legume species *(Medicago polymorpha, Trifolium subterraneum, T. balansae, Ornithopus compressus)* indicated under low P fertilizer levels demonstrated that increases in seed P concentration was associated with increased plant yields (Bolland and Paynther, 1990).

Brachiaria species are the most widely planted forage grasses worldwide, and are well adapted to low P soils. In addition to a root system efficient in P acquisition, this species also uses P efficiently in the plant, as seen in the cultivar Mulato. P use efficiency appeared to be related to enhanced sugar catabolism and increased phosphohydrolase activity in low P conditions, which allows rapid turnover of P (Nanamori et al., 2004).

The QTL for phosphorus uptake and use efficiency were each found on chromosomes 2 and 12 of the rice genome, with the P uptake QTL coming from one parent and the use efficiency coming from the other parent used in the study. While P uptake was positively correlated with plant dry weight, use efficiency was negatively correlated. This indicates that P use efficiency may not be a positive adaptation worth exploring by rice breeders, but may be a response of plants with poor P uptake (Wissuwa et al., 1998).

Summary

The use of root properties as selection criteria to improve P use efficiency through plant breeding requires a trait that is easily identifiable, for which large genetic variability exists and the mode of inheritance is known (Gahoonia and Nielsen, 2004). This poses a challenge using root traits in breeding as characterization is notoriously difficult below ground. Success in breeding rice to be more tolerant to low P soils was attained through a QTL for variation in P uptake. The trait considered in that study was simply plant P concentration; no root system measurements were taken. This QTL was transferred from one

cultivar to another and the resulting near isogenic line more than tripled its grain yield (Wissuwa et al., 2001b).

The same success with breeding for improved tolerance to low P soils has yet to be realized in common bean, despite the large body of literature regarding potential mechanisms of P efficiency and genetic control of related traits. One should keep in mind that seed yield is the bottom line in improving common bean germplasm for tolerance to low P soils. Singh et al. (2003) advocated field selections under general low soil fertility instead of just low soil P. In addition, and in direct contrast to previous finding by Yan et al. (1995a, 1995b) when field selections of more than 5000 landraces and improved common bean lines were conducted based on yield, it was Mesoamerican, not Andean races that were identified as better adapted to low soil fertility (Singh et al., 2003). The discrepancies observed most likely arise from what was measured as an indicator of P efficiency, overall yield or plant P uptake.

Biotic Stress: Fusarium Root Rot

In contrast to the large amount of research that relates low soil phosphorus tolerance to root architectural traits, there has been limited research that associates root architecture with tolerance to Fusarium root rot. There have been numerous studies illustrating that the severity of Fusarium root rot is highly influenced by environmental stresses that impact root growth. This is consistent with an important role for root architecture in determining resistance.

Nature of disease:

Root rot, caused by a combination of fungi, is a major limiting factor in the production of *Phaseolus vulgaris* (Abawi and Pastor Corrales, 1990). The complex of pathogens believed to contribute to root rot include: *Fusarium solani* f.sp. *phaseoli*, *Rhizoctonia solani* Kuhn, *Pythium ultimum*, *Thielaviopsis basicola* (Berk. & Br.) Ferr. and *Aphanomyces euteiches* f.sp. *phaseoli*. In the majority of dry bean and snap bean producing regions of the United States, including Michigan, *F. solani* f.sp. *phaseoli (Fsp)* is considered the major pathogen of the complex (Chatterjee 1958; Keenan et al., 1974; Burke and Kraft 1974; Steadman et al., 1975; Saettler 1982). Yield losses of up to 84% have been attributed to Fusarium root rot (Keenan et al., 1974).

Susceptibility to *Fsp* is developmentally regulated in beans. *Fsp* is not able to effectively cause infection until the plant develops a root system (10 days after planting). Earlier in development, considerably fewer spores attach to plant tissue than in older bean plants. Disease symptoms in younger plants are limited to a hypersensitive response at the inoculation site by the outermost cell layers (Vogeli-Lange et al., 1995). Symptoms of Fusarium root rot include red longitudinal lesions on the hypocotyls, taproot and lateral roots of the bean plants, followed by root rot. In severe infections complete rotting of a root system is observed (Schneider and Kelly, 2000). Increased adventitious rooting is also often a sign of disease infection (Chatterjee 1958; Steadman et al., 1975). Severity of *Fsp* increases as the plant develops, and with time it becomes possible to see symptoms on upper parts of the plant including chlorosis, stunting, and

premature defoliation, along with reduction in pod production (Burke and Barker, 1966).

Although *Fsp* infection is readily visible on the hypocotyl and taproot, lateral root infection most significantly impacts crop yield. A plant with a healthy lateral root system can outgrow the detrimental effects of localized hypocotyl and taproot infection (Burke and Barker 1966). The nature of the infection by *Fsp* may be the reason for the importance of lateral roots. Infection is defined as direct penetration of fungal hyphae into healthy epidermis, wound sites on hypocotyl or root tissue, or through hypocotyl stomata. The hyphae spread extracellularly in the cortex until cell death occurs, at which time hyphae begin to spread intracellularly. The pathogen is unable to pass the endodermis of the taproot, but can enter the vascular system of lateral root during their emergence (Christou and Snyder, 1962) suggesting that large loss of lateral roots leads to the plant's inability to transport enough water and nutrients to the shoot, but this may not be a factor with taproot infection because the vascular system is able to remain functional. The disease cycle is shown in Figure 3.

Environmental Factors:

Under optimal growing conditions, *Fsp* is a minor pathogen of beans. Environmental conditions that increase the stress on plant roots and impede root growth, including drought, flooding, (Miller and Burke, 1977) soil compaction (Burke 1968; Miller and Burke, 1985), low soil fertility, low soil temperature (Burke et al., 1980), and plant competition (Burke, 1965) exacerbate incidence of Fusarium root rot.

Soil temperature

Soil temperature plays an important role in root rot disease incidence caused by *Fsp* on common bean. Soil temperatures fluctuate seasonally and are impacted by air temperature, with the lowest average soil temperatures occurring early and late in the growing season. Common bean grows best at soil temperatures of 22 to 26°C. Temperatures above 30°C cause an inhibition in root growth by reducing cell division and root elongation. Temperatures below 18°C also significantly reduce yield. Chilling temperatures reduces membrane permeability and decrease the ability of the root system to uptake water. Studies conducted by Reddick (1917) and Sippell and Hall (1982a) indicated *Fsp* has a temperature tolerance of 12 to 35°C. Disease caused by *Fsp* appears to be favored by temperatures in the range of 16 to 22°C as compared to warmer temperatures (26 to 34°C). At temperatures above 30 °C there is reduced attachment of fungal spores to the root surface which can decrease amount of infection and disease severity.

Suboptimum soil temperatures for bean growth increase Fusarium root rot incidence. Suboptimal soil temperatures cause decreased root growth, water uptake, and nutrient uptake. Such conditions exacerbate root rot caused by *Fsp* by limiting root growth which reduces a plant's ability to compensate for a portion of the root system malfunctioning from rot. Beans planted in warm soils are often able to escape the yield depressing effects of root rot that occur under cold soil temperatures. There is also an interaction between moisture, temperature, and root rot severity. Although moist soil is most favorable for germination of resting *Fsp* spores, it also favors the growth of the bean seedling and incidence of disease

is much greater in dry soils. In dry soils there is a larger temperature range over which infection occurs as compared to moist soils. (Buerkert and Marschner, 1992). Under low soil temperatures (21°C days 16°C nights) root growth was more reduced in the presence of *Fsp* as compared to inoculum free soil (Burke et al., 1980).

Drought

Deep root systems appear to be an important factor in enhancing resistance to Fusarium root rot under dryland conditions. *Fsp* spores most widely inhabit soil at depths of 15-45 cm below ground, as do the majority of bean roots. Dryden and Van Alfen (1984) found lower levels of *Fsp* infection on roots growing below 45 cm as compared to those roots above 45 cm. Under dryland conditions, soil water availability is often greatest below 60 cm, therefore, deep rooted cultivars may tolerate drought by accessing water 45 to 60 cm below the soil surface. Intact deep roots which are able to take up water may help plants avoid *Fsp* infection (Miller 1985; Burke and Miller, 1983).

Compaction

Soil compaction is an increase in soil bulk density and a decrease in air filled pores as a result of force applied to the soil (Allmaras et al., 1988). Excessive soil compaction, which impedes root growth and function, may be observed at a critical penetration resistance of 2 MPa and a D:Do ratio (oxygen diffusion coefficient in the soil relative to air) of zero at 10% air-filled porosity (Allmaras et al., 1988). Such soil compaction levels are frequently present in agricultural fields as a result of machinery used for planting, harvest, and other

crop maintenance activities (Harverson et al., 2005). Plant root systems respond to increases in soil bulk density or mechanical impedance by exerting a growth pressure to get through soil channels that are smaller than the root diameter (Clark et al., 2003). There is a positive correlation between root diameter and elongation rate under impeded conditions among different species. In addition cultivar differences for ability to penetrate soil with high bulk density have been shown in rice using a wax layer technique in a greenhouse study (Yu et al., 1995). Excessive soil compaction deprives roots of oxygen and they must undergo anaerobic respiration, this process produces less than half the energy of aerobic respiration and halts root growth and maintenance (Allmaras et al., 1988).

Grimes et al. (1975) observed in corn and cotton that in the presence of a compacted layer 30 cm below the soil, roots proliferate above the compacted layer. Asady et al. (1984) developed a controlled environment method to test bean root tolerance to compacted soil and showed that it was strongly correlated (R = 0.91) to field tests. Fewer roots were able to penetrate compacted soil layers, causing an increase in root mass above the compacted layer and an overall reduction in root biomass.

Excessive soil compaction can slow root tip advance up to 75%, making interception and infection by soil fungal pathogens much more likely than in non-compacted soils (Huisman 1982; Allmaras et al., 1988). Studies with bean and *Fsp* showed that inoculation reduced root ability to penetrate a compacted soil layer. Fusarium species are generally likely to infect the hypocotyl or more stationary parts of the root system because they do not germinate quickly and

usually exist largely as colonies epiphytic on the root surface (Allmaras et al., 1988). In compacted fields with Fusarium spores present, tillage increased bean seed yields by 40% and reduce disease severity scores from 3.4 to 1.4 over non-tilled fields (Harverson et al., 2005).

Bean genotypes that perform well in the presence of *Fsp* inoculum also often demonstrate tolerance to other soil stresses. For example the resistant genotype NY-2114-12 was able to increase root growth above the point of soil compaction to a greater extent than susceptible cultivars (Miller, 1985). The resistant snap bean 'FR266' also has been shown to have high tolerance to compacted soil (Silbernagel, 1990). NY 2114-12 also had higher shoot and root growth under water deficit as compared to susceptible cultivars (Miller 1985). Fusarium root rot resistant cultivars outperform susceptible cultivars under each of the environmental stresses listed above except flooding stress. Resistant cultivars were unable to overcome disease under low soil oxygen stress (Miller 1985; Burke and Miller, 1983).

Methods of Control:

Root rot severity in beans is related to environmental conditions that limit root growth. Cultural practices that allow the development of a vigorous root system are essential to reducing root rot, especially when resistant cultivars are unavailable. Thus, planting in warm soil and maintaining optimum soil water are important to establish vigorous root growth which allows plants to better withstand root rot. Irrigation is especially important in dry years because water stressed plants are more susceptible to root rot than non water stressed plants.

Increasing the spacing between plants within rows and between rows may reduce root rot severity by reducing competition among root systems for water and nutrients.

Crop rotation: Crop rotation can be used to reduce *Fsp* populations in the soil. Soil population density (cfu/g) of *Fsp* is highest in continuous bean production and rotation can reduce the amount of inoculum in the soil. *Fsp* is very immobile in the soil with 14 years of continuous corn, soybean or fallow having very little Fusarium (Sippell and Hall 1982b). Recommendations are generally for 4 year rotations to reduce inoculum.

Rotation with small grains and alfalfa add residue to the soils and can reduce compaction, improve the soil structure and increase water holding capacity of the soil. However, residues must be incorporated at least one month before planting or phytotoxicity can result (Burke and Miller, 1983). Organic amendments can also increase overall microbial population in the soil which in some cases reduce habitat and nutrient availability in soil which could reduce competitiveness of *Fsp*.

Deep Tillage: Deep tillage of the soil has been very effective to reduce root rot severity in compacted soils. Both susceptible and resistant cultivars exhibit decreased root rot severity with deep tillage (Tan and Tu, 1995). Deep tillage in the presence of *Fsp* inoculum improves plant yields more so than in non-infested soil. A study by Miller and Burke (1986) found that susceptible cultivars showed a larger yield increase from tillage than resistant cultivars illustrating the importance of deep root growth to overcome infection.

Chemical Treatments: Seed treatments are generally not effective against Fusarium root rot (Sippell and Hall, 1982b). Soil fumigants such as chloropicrin and methyl bromide may suppress Fusarium root rot but are economically and environmentally impractical (Burke and Miller, 1983).

Genetics:

Sources of Resistance:

N203 (PI203958) is considered the best source of resistance to Fusarium root rot in *P. vulgaris*. Although this accession does not exhibit complete immunity, it does have a high degree of resistance. N203 is a late maturing, indeterminate accession collected in Mexico (Wallace and Wilkinson 1965). *P. coccineus*, also a primary source of resistance has shown slightly higher levels of resistance that N203 (Wallace and Wilkinson 1965). Both principle known sources of resistance, N203 and *P. coccineus*, have been reported to exhibit higher levels of resistance in the field as compared to the greenhouse (Wallace and Wilkinson, 1965; Baggett and Frazier, 1959), suggesting root avoidance or escape of infection may be an important component to resistance in the field.

The first Fusarium root rot resistant dry bean cultivars were released in 1974. They included the pink cultivars 'Viva', and 'Roza' and the small red cultivar 'Rufus' (Boomstra and Bliss, 1977). Each cultivar derived resistance from N203 (Burke, 1982). The first snap bean cultivars with resistance to Fusarium root rot were released in 1978. The source of resistance of these cultivars (RRR 77) and (RRR 83) was not specified (Hagadorn and Rand, 1978). Available genotypes with the highest level of resistance are late maturing with a

vine growth habit. It has been difficult to transfer resistance to early maturing short-vine and bush type beans (Burke and Miller, 1983). 'FR266' is a resistant snap bean with 'Blue Lake' pod quality characteristics released in 1987 which derived resistance from N203 and *P. coccineus* (Silbernagel, 1987). It is one of the few genotypes available with Fusarium root rot resistance and a bush type growth habit.

Inheritance of Resistance:

Bravo et al. (1969) found the resistance from N203 and *P. coccineus* to be inherited in a similar manner. They found resistance with either of the parents to be mostly dominant and controlled by 3 or more genes (Bravo et al., 1969). Based on comparisons of the inheritance of resistance in N203 and 2114-12 (resistance from *P. coccineus*), Hassan et al. (1971) found N203 to possess 4 resistance genes and 2114-12 to posses 5-6 resistance genes, with 4 of the resistant genes in 2114-12 also present in N203. Hassan et al. (1971) used a hypocotyl disease severity index to rate disease and did not consider root rot. Thus, their results may not be applicable to 'root rot'. Hassan et al (1971) obtained similar results as Bravo et al. (1969), who scored disease index on the roots. Overall there are many conflicting reports on the number of genes controlling resistance and it is clearly quantitatively inherited. The exact number of genes controlling the trait remains unclear. Numerous conflicting reports on the inheritance of resistance to *Fsp* suggest that growth and environmental conditions influence study results (Boomstra and Bliss 1977).

Schneider et al. (2001) observed while studying a population of RILs derived from a cross between FR266 and Montcalm that there is no hypersensitive response in the resistant genotype, only a slowing of symptom development. In the same population QTL were found to be associated with resistance in the field or in the greenhouse but there was no overlap. In addition, no single QTL explained more than 15% of phenotypic variation, indicating that many genes are involved. Intermediate heritability scores also indicated a high effect on the environment for this trait. In a recent QTL study by Roman-Aviles and Kelly (2005) an inbred backcross line population was used to reduce the background effects and isolate more thoroughly the genetic mechanism for resistance. In this study a total of 5 QTL on two linkage groups explained 73% of the phenotypic variation. Again, QTL identified in field were different than those in the greenhouse. This may be in part because greenhouse plants were scored after two weeks whereas in the field they were scored after flowering or at maturity, resulting in selection for early expressed genes for resistance. One QTL was linked to a marker by Schneider et al (2001) that was identified (FR266 x Montcalm) as important for resistance.

Root Architecture and Disease:

Root pathogen interactions are largely dependent on root growth, as roots are typically much more mobile in the soil than fungal pathogens. Growth rates of root length can serve as an indicator for the potential for root pathogen contact (Huisman, 1982). Root length also serves to indicate how much area is available for water and nutrients in the presence of root rot (Burke and Barker, 1966).

Genotypic variability for root length has been shown to be associated with resistance in common bean. Under field conditions, in the presence of *Fsp* spores, the root rot resistant genotype FR266 had a root dry weight twice that of the susceptible cultivar 'Montcalm', along with a greater length and surface area, indicating an overall larger root system (Roman-Aviles et al., 2004). In addition average root system diameter was negatively correlated with root rot severity (r^2= 0.4) in RILs developed from a cross between Montcalm and FR266. The average number of adventitous in the top cm was positively correlated with root rot tolerance (r^2=0.6) (Snapp et al., 2003). In a screening of 10 bean cultivars from different market classes and with varying susceptibility to Fusarium root rot the three most resistant cultivars also had the most adventitious root mass (Roman-Aviles et al., 2004). Adventitious roots are commonly considered a host plant response to compensate for the infection of taproots and lateral roots (Chatterjee, 1958; Steadman et al., 1975).

A few studies have examined the role of root system architecture in resistance to root pathogens. Vine decline of muskmelon (*Cucumis melo* L.) is caused by a complex of soil borne fungi, the most prominent fungus, *Monosporascus cannonballus* causes the root rot symptoms. As with Fusarium root rot in bean, disease severity is strongly influenced by the environment and no highly resistant cultivars exist (Dias et al., 2002). Root morphology variability has been discovered in tolerant and susceptible cultivars infected with the pathogen. Tolerant cultivars had more vigorous root systems, including a greater total root length, more root tips, and a greater fine root length than susceptible

cultivars (Crosby et al., 2000). The highly resistant wild melon accession Pat81 had a larger root system and more lateral roots that a highly susceptible commercial cultivar 'Amarillo Canario' both in the presence and absence of fungal inoculum (Dias et al., 2002). Pat81 was used as a parent in a backcross breeding program, and progeny with larger root systems demonstrated root rot tolerance (Dias et al., 2004).

Root and Shoot Growth Habit Coordination

Common bean shoot architecture has been classified into four types: determinate (Type I), indeterminate upright (Type II), indeterminate prostrate (Type III), and vine (Type IV) (Kelly 2001). Limited observational studies support a coordinated growth habit of the root and shoot (Lynch and van Beem 1993; Kelly, 1998). 'Carioca' a type III plant with highly branched shoot architecture and little apical dominance also exhibited highly branched root architecture (Lynch and van Beem 1993). In the case of Fusarium root rot resistance, it has been much easier to develop resistance in indeterminate type cultivars than in determinate cultivars. Therefore, if root architecture is important for resistance to Fusarium root rot, it may be strongly influenced by shoot architecture traits such as total leaf area. To effectively breed determinate Fusarium resistant bean cultivars an understanding of root and shoot traits is required to determine how to separate them.

Summary:

A half century of research has shown that Fusarium root rot severity in common bean is strongly influenced by environmental conditions that affect plant root growth. In addition all known sources of resistance provide partial resistance to infection. These factors of disease have indicated that chemical resistance is not a reliable selection tool as seen with differences between genetic control of field and greenhouse resistance. Breeding of cultivars resistant to *Fsp* is typically done in combination with breeding for general tolerance to stress.

Only within the last few years have researches began to study the relationship between root architecture and Fusarium root rot. In that time, it has been shown that indeed, the resistant cultivars do have larger root systems in field conditions than susceptible cultivars. Much more research needs to be conducted, however to determine the genetic control of this relationship and what root traits may serve as selection tools to facilitate breeding efforts that can provide cultivars that are less susceptible to Fusarium root rot in the presence of environmental stresses.

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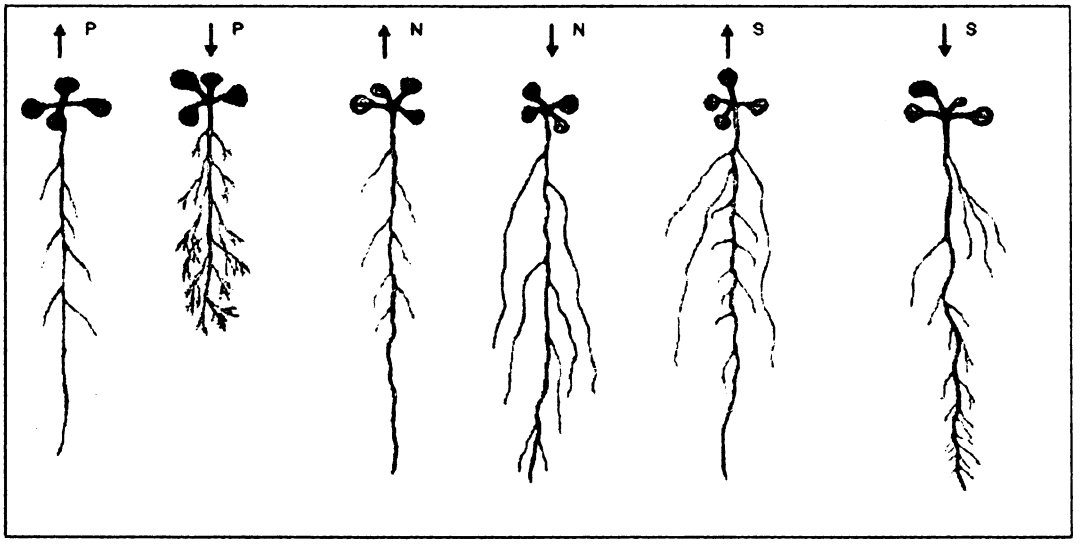

Figure 1. Responses of Arabidopsis root systems to supply of nutrients P, N, and S. Figure reproduced from Lopes-Bucio et al. (2003).

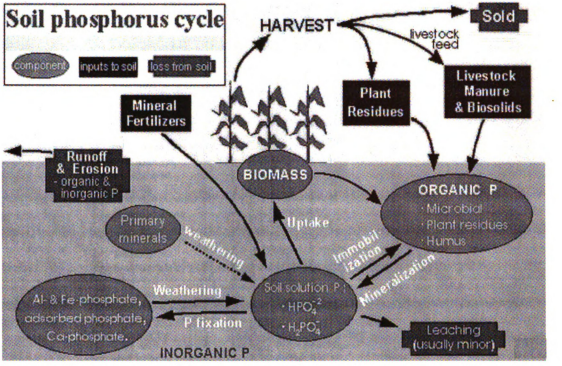

Figure 2: The Phosphorus Cycle (Potash and Phosphate Institute, 2004)

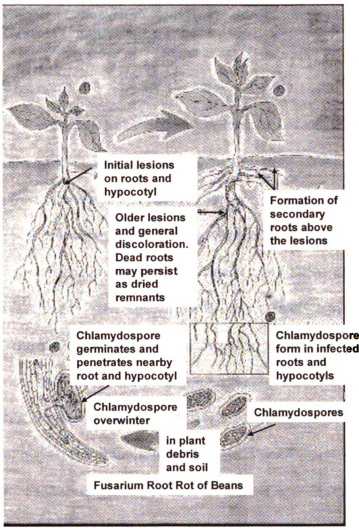

Figure 3: Fusarium root rot infection in beans (Schwartz, 2006)

Chapter 2: Fusarium Root Rot Incidence and Root System Architecture in Grafted Common Bean Lines

Abstract

There is minimal understanding of the complex genotype by environment interaction controlling Fusarium root rot (caused by *Fusarium solani* f.sp. *phaseoli*) disease expression in common bean (*Phaseolus vulgaris* L.). Severity of the disease is increased by environmental factors such as soil compaction that add additional stress to the plant root system. It has proven difficult to develop resistant cultivars, especially with a bush growth habit as seen in snap and kidney bean market classes. One resistant determinate snap bean line, 'FR266', has been developed. The current study used reciprocal grafting techniques with resistant 'FR266' and susceptible 'Montcalm' genotypes to determine if the genetic control of resistance lies in the root genotype. The influence of a compacted layer on the root and shoot genotype and root rot resistance was studied. Root rot resistance was found generally to be controlled by the root genotype. However, in the presence of a compacted layer, the root and the shoot genotype play a part in the resistance. Root traits were also observed under these conditions and root mass and root diameter were shown to be controlled by the root genotype without compaction but by the root and shoot genotype when a compacted layer was present.

Introduction

Root rot of common bean (*Phaseolus vulgaris* L.) is caused by a combination of fungi and environmental stress and is a major limiting factor in production (Abawi and Pastor Corrales 1990). The complex of pathogens believed to contribute to root rot include: *Fusarium solani* f.sp. *phaseoli*, *Rhizoctonia solani* Kuhn, *Pythium ultimum*, *Thielaviopsis basicola* (Berk. & Br.) Ferr. and *Aphanomyces euteiches* f.sp. *phaseoli*. In the majority of dry bean and snap bean producing regions of the United States, *F. solani* f.sp. *phaseoli* (*Fsp*) is considered the major pathogen and severe infections have caused yield losses of up to 84% (Chatterjee, 1958; Keenan et al., 1974; Burke and Kraft, 1974; Steadman et al., 1975; Saettler, 1982).

Symptoms of Fusarium root rot include red longitudinal lesions on the hypocotyls, taproot and lateral roots of the bean plants which lead to rot of infected roots. Severity of infection increases as the plant develops, and with time complete rotting of the root system can occur and above ground symptoms develop including leaf chlorosis, stunting of stems, and reduction in pod production (Schneider and Kelly, 2000; Burke and Barker, 1966).

Under optimal growing conditions, *Fsp* is a minor pathogen of beans. Environmental conditions such as excessive soil compaction can cause plant stress and constrain optimal root development and enhance Fusarium root rot development (Burke, 1968). Soil compaction is an increase in soil bulk density and a decrease in air filled pores as a result of force applied to the soil; excessive soil compaction impedes root growth and function (Allmaras et al., 1988). Soil

compaction is frequently present in agricultural fields as a result of the frequent passage of heavy machinery over soil used for planting, harvest, and other crop maintenance activities (Harverson et al., 2005). Excessive soil compaction can slow root tip advance up to 75%, making interception and infection by soil fungal pathogens much more likely than in non compacted soils (Huisman, 1982, Allmaras et al., 1988). In compacted soil in the presence of inoculum of *Fsp*, tillage enhanced bean seed yields by 40% and reduced disease severity scores from 3.4 to 1.4 (on a scale of 0-4, where 0 indicates no disease and 4 indicates more than 75% of the root system is rotted) over non tilled fields (Harverson et al., 2005).

Bean genotypes with the highest level of Fusarium root rot resistance are late maturing with an indeterminate plant growth habit. It has been difficult to transfer resistance to beans with a determinate bush type plant growth habit (Burke and Miller, 1983). FR266, a resistant snap bean was released in 1987 and derived resistance from N203 a late maturing, indeterminate accession collected in Mexico (Wallace and Wilkinson, 1965) and *Phaseolus coccineus* (Silbernagel 1987). FR266 has no hypersensitive response, only a slowing of symptom development. Schneider et al (2001) investigated QTL for resistance in a population of recombinant inbred lines derived from a cross between FR266 and a highly susceptible kidney bean variety 'Montcalm'. QTL were identified, however, no single QTL explained more than 15% of the phenotypic variation and different QTL were identified in greenhouse as compared to field tests. Intermediate heritability scores also indicated a high effect of the environment for

this trait. Breeders have typically not relied only on root rot resistance scores from greenhouse screening alone, but have combined root rot scoring with field screening for stress tolerance that involved compaction and drought interactions (Burke and Miller, 1983).

The effect of environmental factors such as compaction (Miller and Burke, 1985) on disease severity and the necessity to develop stress tolerant plants in general raised the question, what traits besides biochemical resistance may be responsible for the resistance seen in the cultivar FR266. Characteristics such as root system architecture (Snapp et al., 2003) may be involved. Under field conditions, in the presence of *Fsp* chlamydospores, FR266 had a root dry weight twice that of Montcalm, along with a greater length and surface area, indicating an overall larger root system (Roman-Aviles et al., 2004). The average number of lateral roots in the top 1.0 cm of soil was positively correlated (r^2=0.6) with root rot tolerance in snap beans (Snapp et al., 2003). Lateral root density per volume of soil may be predictive of stress tolerance rather than resistance to *Fsp* and has been associated with drought tolerance in lettuce (Johnson et al., 2000). FR266 also has been shown to grow well in compacted soil (Silbernagel, 1990). Previous studies on compaction tolerance in corn (*Zea mays* L.) and soybean (*Glycine max* L.) have identified cultivar specific variability in the ability of roots to grow through compacted layers (Bushamuka and Zobel, 1998). These findings suggest that in compacted soils biochemical resistance to a pathogen is not the only factor required for a plant to grow well.

The difficulty in introducing root rot resistance into Andean beans, has led to the hypothesis that there are inherent physiological factors in Fusarium root rot resistant bean cultivars that are expressed only under field conditions. Root and shoot growth is genetically integrated, therefore determining if a trait such as Fusarium root rot resistance is expressed in the root, the shoot, or the whole plant is difficult to discern in whole plant or hybridization studies, but can be differentiated by interchanging root and shoot genotypes via grafting (White and Castillo, 1989; Izquierdo and Hosfield, 1982).

The grafting technique has been demonstrated to be a sound tool for genetic research in common bean. Research has demonstrated the technique is free of artifacts by employing the self graft as a control for the technique (White and Castillo, 1989; Izquierdo and Hosfield, 1982). Using the reciprocal grafting technique to study drought tolerance in common bean, the root genotype, or more specifically, genes expressed only in the root system, had a large effect on seed yield under drought conditions. The shoot genotype had some effect on the trait, but much less than that of the root (White and Castillo, 1989; White and Castillo, 1992). A study of resistance to the soil borne pathogen, *Phialophora gregata* which causes brown stem rot in soybean was also found to be controlled by the root system (Bachman and Nickell, 1999). Pantalone et al. (1999) found that the root system architecture trait "fibrous-like rooting" was controlled by the root system and enhanced seed biomass when grafted onto some soybean genotypes. In addition there is evidence that the shoot genotype influences the root system

phenotype as seen with nodule development and N fixation in soybean (Sheng and Harper, 1997).

The objectives of this research were to determine a) if the expression of Fusarium root rot resistance was mediated by root or shoot factors and if specific root architectural traits were associated with resistance in reciprocally grafted Fusarium root rot resistant and susceptible bean lines and to evaluate b) how root rot resistance and root architectural traits were mediated by root and shoot factors when reciprocally grafted plants were grown with the added stress of soil compaction.

Material and Methods

Plant material

Two common bean genotypes with differences in susceptibility to Fusarium root rot were used in this study. They were the snap bean line 'FR266' characterized as root rot resistant (Silbernagel, 1987) and the kidney bean 'Montcalm' characterized as root rot susceptible (Schneider et al., 2001). FR266 was developed by USDA/ARS in Prosser, WA (Silbernagel, 1987). Montcalm is representative of commercial kidney bean types and was developed at Michigan State University (Copeland and Erdmann, 1977). Select characteristics of FR266 and Montcalm are described in Table 1.

Reciprocal Grafting

In all experiments reciprocal grafting was employed to determine if genetic control of Fusarium root rot resistance originated from the shoot or root system. Plants were grafted in one of four combinations. The first combination

was FR266 shoot grafted onto Montcalm root and the second combination was Montcalm shoot grafted onto FR266 root. The third and fourth graft types were self grafts of FR266 shoot grafted onto FR266 root and Montcalm shoot grafted onto Montcalm root, respectively The self grafts were used as controls to determine the effect of grafting on root rot and plant growth and these were compared to ungrafted FR266 and ungrafted Montcalm plants also included in all experiments.

Seeds were surface sterilized by soaking 2 min in 70% ethanol, followed by 2 min in 0.5% NaOCl. Seeds were rinsed with distilled water 4 times and incubated at 18°C in the dark in moist germination paper for 2 days. Seeds were planted as described below for each experiment. Five days after germination, seedlings were grafted using the cleft graft technique (Izquierdo and Hosfield, 1982). A razor blade was used to cut through the stem 1.5 cm above the cotyledon. The bottom 1.5 cm of the stem of the shoot was cut into a wedge shape with a razor blade by removing a thin slice of the epidermis on 2 sides of the stem. A longitudinal cut of 1.5 cm was made into the top part of the stem of the root system. The wedge shaped base of the shoot's stem was placed into the stem above the root system. The shoot and root were held together with parafilm.

Grafted plants were kept in a controlled environment at a temperature of 25°C day and a relative humidity of 90%. Plants received 14 hr of light (350-450 mE). Plants were misted with water by hand with a spray bottle every 4 hours until callus tissue formed (5-7 days) and water could be supplied through the root system.

Experiments:

Two distinct controlled environment plant growth experiments were conducted and each experiment was repeated twice. All experiments were set up as randomized complete block designs with two treatments, inoculation with Fusarium solani spores and no inoculation. There were 6 plant genotypes grown under each treatment, including each of the 4 graft types described above and ungrafted plants of FR266 and Montcalm. There were four replications of each treatment/genotype combination.

Experiment 1 Non-compacted soil:

Media: Sand harvested from Sandhill Farm, Michigan State University, East Lansing, MI was sieved, sterilized via autoclaving, and mixed with perlite (60:40) by volume.

Containers: Seeds were planted into 72 cell flats. Five day old plants were grafted as described above. One week following grafting, plants were transplanted into containers referred to as root observation boxes. These containers promoted the plant's root system to grow flat and therefore improved the ability to harvest roots for measurement. The containers were constructed of glass panes 21.6 cm by 27.9 cm separated by 5.1 cm wooden strips for a dimension of 5.1 x 21.6 x 27.9 cm (Figure 1a).

Plant growth conditions: Root observation boxes were completely covered with aluminum foil to prevent light from interfering with root growth. Individual root observation boxes were placed together into the growth chamber with other boxes, each at a 45° angle. Plants were exposed to 14 h of light (350-450 mE) at 28°C

and 10 h dark cycle at 25°C. Plants were watered as needed and were fertilized weekly with ½ strength Hoagland's solution (Hoagland and Arnon, 1938).

Fusarium spore preparation and inoculation: Fourteen day old plants were inoculated with 15 ml of 2.0 x 10^5 conidia ml^{-1} suspension of a mix of 2 isolates of *F. solani* f. sp. *phaseoli* (*Fsp).* The Hawks 2B isolate was collected by Schneider and Kelly (2000) in Presque Isle County, MI and S-602 was received from the Penn State Fusarium Research Center, University Park, PA. Potato dextrose agar (PDA, Invitrogen ™, Life Technologies, MD; 39 g L^{-1} water) was the media used to grow the Fusarium colonies. Inoculum discs, 4 mm diameter were cut from margins of 1- week old cultures grown on PDA and transferred mycelial side down, to the center of individual Petri plates (15 mm depth x 90 mm diameter) containing 25 ml of PDA. Plates were stored at room temperature for 2 weeks prior to inoculum preparation. The inoculum was prepared by scraping mycelium and conidia from PDA plates into distilled water, then strained through cheesecloth. The conidial concentration was adjusted to 2 x 10^5 ml^{-1} using a hemocytometer. Fifteen ml of the inoculum was applied to the soil at the base of the plant.

Harvest: Plants were harvested 32 days after planting and shoots were separated from roots and soil was washed from the root system with distilled water. The experiment was carried out twice, in August 2003 and in August 2004.

Experiment 2: Compacted soil layer:

Media: The growing media was a Capac loam harvested from the Michigan State University Horticulture Farm, East Lansing, MI. Soil was sieved, autoclaved, air dried and brought to 18% moisture before adding to containers.

Containers: PVC tubes of 7.6 cm diameter and 0.5 cm wall thickness were used for plant growth containers based on the design of Asady et al (1985). The containers consisted of three layers. The top layer was 12.5 cm long, the middle layer was 2.6 cm long, and the bottom layer was 15 cm long (Figure 1b). Soil was added to the top and bottom layers to achieve a bulk density of 1.1 g/cm^3. The middle layer was compacted to a bulk density of 1.7 g/cm^3 with a hydraulic compactor machine. Bulk densities of each layer were determined based on soil weight. The bulk density of the compacted layer was chosen because the soil strength at that density reduces common bean root growth (Asady et al., 1985). The three layers of the container were held together with duct tape.

Fusarium spore preparation and inoculation: The same mix of 2 isolates of *F. solani* f. sp. *phaseoli* (*Fsp)* were used in this experiment as described in Experiment 1, Fusarium spore preparation and inoculation. Colonies were first grown on potato dextrose agar (PDA, Invitrogen ™, Life Technologies, MD; 39 g L^{-1} water). After one week of growth, fusarium inoculum discs, 4 mm diameter were cut from margins and transferred mycelial side down to sterile pearl millet seed prepared as follows. Millet seed (1000 ml) was poured in an aluminum pan (30.5 cm x 25.4 cm x 11.4 cm) mixed with 600 ml of distilled water, covered with aluminum foil and was kept at room temperature for 12 h. The pan was

autoclaved for 4 h and cooled 4 h. Next, 12 inoculum disks (1.3 cm wide) were evenly spaced over the seed. Two-week old cultures were used to prepare inoculum. Millet seed was mixed to homogeneity. Mycelium and conidia of a 1 gm sample of the seed was collected in distilled water. The inoculum concentration of the sample was determined with a hemocytometer. The weight of seed for a conidial concentration of 2×10^5 ml^{-1} was determined. The inoculum/millet mixture was combined with the soil for the top layer of the container.

Plant growth conditions: Surface sterilized pregerminated seeds were planted directly into containers, two seeds per container. After germination, seeds were thinned to one per container. Grafting, as described above, was conducted directly in containers. Plants were exposed to 14 h of light (350-450 mE) at 28°C and 10 h dark cycle at 25°C. Plants were watered as needed. Percent moisture of the soil was kept at 18% and the amount of water to add to each plant was determined by container weight. Plants were fertilized weekly with ½ strength Hoagland's solution (Hoagland and Arnon, 1938).

Harvest: Thirty-two day old plants were harvested and shoots were separated from roots. Roots were harvested separately from each of the three layers of the containers and soil was washed from the roots with distilled water. The experiment was carried out once in November 2005. In October, 2004 the experiment was carried out with ungrafted and reciprocal grafts only.

Plant Measurements:

Following harvest of plants in each experiment, root rot scores were measured based on a scale of 1 to 7 as described by Schneider et al (2000) where "1= healthy root system with no discoloration of root or hypocotyls tissue and no reduction in root mass compared to the uninoculated control; 2 =appearance of small reddish-brown lesions, 0.1-0.2 cm in length, at base of hypocotyls with size and appearance of root mass normal; 3 = increase in intensity and size and coalescing of localized root/hypocotyl lesion circling ≈ 180° of stem with lesions from 0.5-1cm and 10% to 20% discoloration on roots but no reduction in size of root mass; 4 =increasing intensity of discoloration and size of hypocotyl lesions with lesions becoming extended and completely encircling the stem; 5% to 10% reduction in root mass with 95% of the roots discolored, 5 = increasingly discolored and extended hypocotyls lesions. 100% of the roots intensely reddish-brown with a 20% to 50% reduction in root mass, 6 = hypocotyl lesions encircling stem become more extended (2 cm); root mass is intensely discolored and reduced from 50% to 80%; 7 = pithy or hollow hypocotyl with very extended lesions, where root mass is reduced from 80% to 100% and is functionally dead."

Following root rot scoring, cleaned roots were placed in a transparent plastic tray (22 x 28 x 6.5 cm) and covered with water. The tray containing roots was then scanned with a flat bed scanner with a top and bottom light source at 300 dpi to produce a 2 dimensional scanned image. The images were analyzed with WinRHIZO software (Regent Instruments Inc., Quebec, Canada). With this program root length, root surface area and average root diameter of the samples

was determined. Following image capture, roots and shoots were dried at 65°C for two days and dry weights were determined.

Data Analysis:

Statistical analysis was conducted with SAS for Windows V8 (SAS Institute, Cary, NC). The command PROC GLM was used to determine treatment, genotype and interaction effects. An initial model with 2 runs, 4 replications, 2 treatments, and 6 genotypes, was run for experiment 1 to determine the effect of Fusarium inoculation and grafting on plant growth. The same model was developed for experiment 2, but there was 1 run, instead of 2 runs in the model. A second model was then developed with 2 runs (1 run for experiment 2), 4 replications, 1 treatment (inoculated plants only) and 2 shoot genotypes (FR266 or Montcalm) and 2 root genotypes (FR266 or Montcalm. Ungrafted plants and uninoculated plants were excluded from this model. This model was used to determine the specific effects of each root and shoot genotype in the presence of Fusarium inoculum with out confounding results with ungrafted plants. Least significant differences (LSD) values were used to determine significant differences among treatments.

Results:

Experiment 1: Consistent with earlier results (Schneider et al., 2001), ungrafted Montcalm plants grown in the presence of *Fsp* spores had a significantly higher root rot score than ungrafted FR266 plants (Table 2). No significant differences were observed between FR266 and Montcalm for root dry weight in the presence of *Fsp* inoculum, which is not unexpected based on length

of experiment. Root measurements were taken on 32 day old plants, and at this time root rot symptoms of root lesions and discoloration were visible, but a longer growth period would be required to observe differences in growth response. Similar results have been observed by other researchers (Roman-Aviles et al., 2004).

Root system architectures of FR266 and Montcalm were characterized by root weight, length, and diameter. The most striking difference between FR266 and Montcalm root architecture was average root diameter. In both inoculated and uninoculated plants, FR266 had a greater average root diameter than Montcalm (Table 3).

The reciprocal grafting technique was used with FR266 and Montcalm plants to understand genetic control of resistance to Fusarium root rot and root architecture. Self grafts of each genotype were included and compared to the ungrafted genotypes as controls to determine if the technique itself impacted reaction to Fusarium. The ungrafted and grafted plants did not differ in root rot scores (Table 2). The ungrafted plants exhibited larger root dry weights than their self grafted counterparts (Table 2).

The comparison of root traits in self grafts and reciprocal grafts in the presence of *Fsp* inoculum showed that root rot score is controlled by the root genotype (Table 4) and it was the FR266 root in any combination that exhibited the lower score (Tables 4 and 5). Root dry weight was also controlled by the root genotype (Table 4) and the FR266 root in any graft combination produced a larger root system that the Montcalm root (Table 5). Average root diameter was

controlled by the shoot genotype (Table 4). The FR266 shoot in any graft combination increased the average diameter of the root system (Table 5). For each of the traits described above, there were no significant root*shoot interactions, which allowed for the presentation of data averaged over root or shoot genotype as in Table 5.

Experiment 2: Ungrafted Montcalm plants had higher root rot incidence than FR266 plants when they were grown in containers with a compacted soil layer (1.7 g /cm^3) at 12.5 cm below the surface of the container (Table 6).

Differences in root architecture between FR266 and Montcalm were observed in the soil below the compacted layer. In the presence of *Fsp* inoculum, FR266 plants had greater total root length and average root diameter than Montcalm plants (Table 7).

Comparison of ungrafted and self grafted plants indicate that grafting did not affect root rot scores (Table 7). Grafting, however, reduced shoot and root dry weight of both FR266 and Montcalm plants (Table 7).

Root rot incidence of grafted plants in this experiment was affected not only by the root genotype as it was in Experiment 1, but was affected by root and shoot genotype (Table 8). The graft combination FR266 root with Montcalm shoot had the same root rot incidence as the graft combination Montcalm root with FR266 shoot.

The root architecture variables root dry weight, root length, and average root diameter also were influenced not by root or shoot genotype alone, but by the interaction of root and shoot genotypes (Table 8). The graft combination

Montcalm root/FR266 shoot, which had a different reaction to root rot in this experiment than in Experiment 2, also exhibited greater root dry weight and root length in the middle compacted and bottom layers of soil than the Montcalm self graft (Table 8). Ungrafted and self grafted Montcalm plants exhibited reductions in root length below the compacted soil layer in the inoculated treatment (Tables 7 and 8) not exhibited by the Montcalm root/FR266 shoot grafted plants (Table 8).

The Montcalm root/FR266 shoot graft combination did not, however, have a larger average root diameter than the Montcalm self graft. Each of the four graft combinations had consistently similar root diameters in this experiment, except in the top layer where the Montcalm root/FR266 shoot combination had a lower average root diameter (Table 8).

Discussion:

Experiments 1 and 2 showed that root rot incidence was controlled by genes expressed only in the root system in the absence of a compacted layer and by genes expressed throughout the plant in the presence of a compacted layer. These results demonstrated that different mechanisms of resistance to *Fsp* were at work under different environmental conditions. I looked at a few basic root architectural traits in each experiment to determine their involvement in *Fsp* resistance.

One of the potential *Fsp* resistance mechanisms of FR266 is its thick roots (Snapp et al., 2003). The reciprocal grafting technique allowed the testing of the hypothesis that thicker roots have less root rot incidence than thinner roots. In Experiment 1, it was found that the root genotype dictated root rot score, whereas

the shoot genotype dictated average root diameter. Since the graft combination Montcalm root/FR266 shoot had thicker roots than the Montcalm self graft, but did not have reduced root rot incidence, therefore root thickness was not a factor in root rot resistance under the experimental conditions. It would be interesting to determine if the same relationship holds true later in plant development when root rot symptoms are more severe.

In Experiment 2, there were no clear differences in root diameter between the genotypes. In the compacted layer there was a uniform reduction of average root diameter of each graft combination, indicating both FR266 and Montcalm responded in the same way to compaction. Small diameter roots have the ability to grow through smaller pores in compacted soils than larger diameter roots (Bennie, 1991). This may be the reason why a reduction in average root diameter was observed in the compacted layer. Average root diameter was influenced by the root and shoot genotypes. Gibberellin (GA) may be a possible signal from the shoot that influenced average root diameter in this study. Gibberellin moves from shoot to root and vice versa and inhibitors of GA biosynthesis have been shown to increase root diameter (Tanimoto, 2005).

Total root length was not an indicator of root rot resistance in Experiment 1. In Experiment 2, however, increased root length below the compacted layer was observed in the resistant genotypes and graft combinations, indicating that FR266 (in any root or shoot combination) was better able to penetrate the compacted layer than Montcalm. One of the mechanisms by which roots move through soils with an increased bulk density and a smaller pore size is by

increasing root diameter and displacing soil to push through an area of compaction (Goodman and Ennos, 1999).

The different interaction of root and shoot signals under varying soil environmental conditions is a likely reason for the difficulty in developing common bean lines tolerant to Fusarium root rot.

Conclusions

Root genotype controlled root rot incidence in the absence of compaction, but with the addition of a compacted soil layer, the interaction of the root and shoot genotype dictated root rot incidence. In the absence of compaction, root traits were clearly influenced by root or shoot genotype. In the soil with a compacted layer, interaction of the root and shoot genotypes was important in determining root system architecture.

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Table 1. Selected plant and seed characteristics of the common bean cultivars FR266 and Montcalm.

	Cultivars	
	FR266	**Montcalm**
Reaction to *Fusarium solani*	resistant	susceptible
Gene pool	Andean	Andean
Market class	Snap	Kidney
Plant growth habit	determinate	determinate
Seed color	white	red
Seed size	medium	large
100 seed weight	30.7 g	61.3 g

Table 2. Mean response root rot score, shoot dry weight, and root dry weight of different graft combinations of common bean lines FR266 and Montcalm in the presence (+ Fus) or absence (-Fus) of *Fusarium solani* f.sp *phaseoli* inoculum. Data analyzed across 2 runs of experiment 1.

		Root rot score (1-7) †		Shoot dry weight (mg)		Root dry weight (mg)	
Graft combination							
Root	**Shoot**	**-Fus**	**+Fus**	**-Fus**	**+Fus**	**-Fus**	**+ Fus**
FR266	ungrafted	1.5	2.9	785	834	604	580
FR266	FR266	1.0	2.1	634	718	330	413
FR266	Montcalm	1.4	2.5	726	831	470	526
Montcalm	ungrafted	1.6	4.1	770	868	449	530
Montcalm	Montcalm	1.8	4.5	816	782	381	371
Montcalm	FR266	1.6	4.3	705	710	459	408
LSD 0.05‡		0.84		172		132	

ANOVA

Source	**df**		**p value**	
Run (R)	**1**	0.0001	0.0006	<0.0001
Replication	**3**	0.8838	0.0649	0.8174
Graft (G)	**5**	<0.0001	0.0461	<0.0001
Fusarium (F)	**1**	<0.0001	0.1068	0.3075
G*F	**5**	0.0018	0.7542	0.3324
R*G	**5**	0.5961	0.4347	0.1013
R*G*F	**5**	0.3825	0.1603	0.0264

† Root rot score is on a scale of 1-7, where 1 is no root rot and 7 is completely rotted (Schneider and Kelly, 2000).

‡ LSD value to compare any values within root rot score, shoot dry weight, and root dry weight variables.

Table 3: Mean response for total root length and average root diameter of ungrafted Montcalm and FR266 in the presence (+ Fus) or absence (-Fus) of *Fusarium solani* f.sp *phaseoli* inoculum. Data analyzed across 2 runs of experiment 1.

Genotype	Total root length (cm)		Average root diameter (mm)	
	-Fus	+Fus	-Fus	+Fus
FR266	3475 a†	3402 a	0.461 a	0.467 a
Montcalm	3273 a	2990 a	0.417 b	0.399 b

†= Values that do not share a letter are significantly different as determined by LSD (0.05). Letters are compared down a column.

Table 4: Mean response and analysis of variance for root traits of different graft combinations of common bean lines FR266 and Montcalm in the presence of *Fusarium solani* f.sp *phaseoli* inoculum. Data analyzed across 2 runs of experiment 1 and the category graft combination is separated into root and shoot components for analysis to determine which components are affecting a response.

Graft Combination					
Root	**Shoot**	**Root Rot Score (1-7)†**	**Root dry wt. (mg)**	**Root length (cm)**	**Avg root diam. (mm)**
FR266	FR266	2.1 a‡	453 ab	3070 ab	0.45 a
FR266	Montcalm	2.5 a	527 b	3209 b	0.44 a
Montcalm	FR266	4.3 b	351 a	2920 ab	0.46 a
Montcalm	Montcalm	4.5 b	371 a	2689 c	0.40 b

ANOVA					
Source	**df**	**p value**			
Run (R)	1	<.0001	<.0001	0.04	<.0001
Replication	3	0.40	0.95	0.18	0.17
Root	1	<.0001	0.01	0.17	0.23
Shoot	1	0.26	0.23	0.86	0.04
R*Root	1	0.82	0.18	0.67	0.29
R*Shoot	1	0.26	0.11	0.47	0.98
Root*Shoot	1	0.82	0.34	0.42	0.13
R*Root* Shoot	1	0.26	0.75	0.81	0.61

† Root rot score is on a scale of 1-7, where 1 is no root rot and 7 is completely rotted (Schneider and Kelly, 2000).
‡ Values that do not share a letter are significantly different as determined by LSD (0.05). Letters are compared down a column.

Table 5: Mean response for root traits of different graft combinations of common bean lines FR266 and Montcalm in the presence of *Fusarium solani* f.sp *phaseoli* inoculum. Data analyzed across 2 runs of experiment 1 and the category graft combination is separated into root and shoot components for analysis to determine which components are affecting a response, means are averaged by root genotype and shoot genotype.

Root †	**Root Rot Score (1-7) ***	**Root dry wt. (mg)**	**Root length (cm)**	**Avg. root diam. (mm)**
FR266	2.31 a**	492 a	3144 a	0.446 a
Montcalm	4.38 b	362 b	2805 a	0.426 a
Shoot ‡				
FR266	3.19 a	402 a	2991 a	0.455 a
Montcalm	3.50 a	449 a	2949 a	0.418 b

† Root heading indicates that means of each variable are averaged over root genotype.

‡ Shoot heading indicates that means of each variable are averaged over shoot genotype.

* Root rot score is on a scale of 1-7, where 1 is no root rot and 7 is completely rotted (Schneider and Kelly, 2000).

** Values that do not share a letter are significantly different as determined by LSD (0.05). Letters are compared down a column; comparisons under 'Root' are separate from those under 'Shoot'.

Table 6. Mean response of root rot score, shoot dry weight, and root dry weight of different graft combinations of common bean lines FR266 and Montcalm grown in containers with a layer of compacted soil in the presence (+ Fus) or absence (- Fus) of *Fusarium solani* f.sp *phaseoli* inoculum, Data analyzed across 1 run (Nov. 2005) of experiment 2.

		Root rot score (1-7) †		Shoot dry weight (mg)		Root dry weight (mg)	
Graft combination							
Root	**Shoot**	**-Fus**	**+Fus**	**-Fus**	**+Fus**	**-Fus**	**+ Fus**
FR266	ungrafted	1.0	2.4	1424	1436	350	398
FR266	FR266	1.0	2.3	717	647	129	153
FR266	Montcalm	1.0	2.5	710	651	140	144
Montcalm	ungrafted	1.0	4.0	1158	932	198	256
Montcalm	Montcalm	1.0	4.5	611	321	129	115
Montcalm	FR266	1.0	2.5	700	692	110	129
LSD 0.05‡		0.74		252		94	

ANOVA				
Source	**df**	**p value**		
Replication	**3**	0.5330	0.2752	0.8888
Graft (G)	**5**	<0.0001	<0.0001	<0.0001
Fusarium (F)	**1**	<0.0001	0.0230	0.2062
G*F	**5**	<0.0001	0.3361	0.8516

† Root rot score is on a scale of 1-7, where 1 is no root rot and 7 is completely rotted (Schneider and Kelly, 2000).

‡ LSD value to compare any values within root rot score, shoot dry weight, and root dry weight variables.

Table 7: Mean response for total root length and average root diameter of ungrafted Montcalm and FR266 grown in containers with a layer of compacted soil in the presence (+ Fus) or absence (-Fus) of *Fusarium solani* f.sp *phaseoli* inoculum, Data analyzed across 2 runs of experiment 2.

Soil layer	Genotype	Total root length (cm)		Average root diameter (mm)	
Top		**-Fus**	**+Fus**	**-Fus**	**+Fus**
	FR266	1577 a†	677 a	0.454 a	0.43 a
	Montcalm	1412 a	525 a	0.419 b	0.42 a
Middle					
	FR266	181 a	215 a	0.380 a	0.37 a
	Montcalm	248 a	180 a	0.344 a	0.37 a
Bottom					
	FR266	2483 a	4322 a	0.406 a	0.45 a
	Montcalm	2141 a	3082 b	0.376 a	0.40 b

†Values that do not share a letter are significantly different as determined by LSD (0.05). Letters are compared down a column.

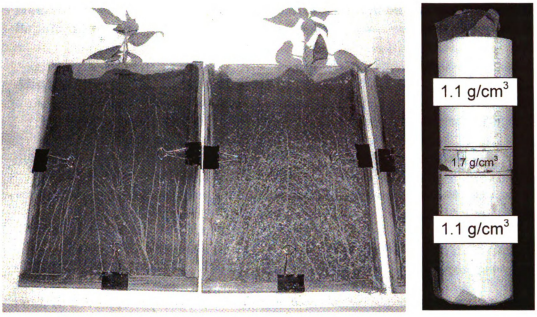
Table 8. Mean response and analysis of variance for root traits of different graft combinations of common bean lines FR266 and Montcalm grown in containers with a layer of compacted soil in the presence of *Fusarium solani* f.sp *phaseoli* inoculum. Data analyzed across 1 run (Nov. 2005) of experiment 2 and the category graft combination is separated into root and shoot components for analysis to determine which components are affecting a response.

	Graft combination					
Soil layer†	**Root**	**Shoot**	**Root Rot Score (1-7)‡**	**Root dry wt. (mg)**	**Root length (cm)**	**Avg root diam. (mm)**
Top	FR266	FR266	2.25 a *	89 a	461 a	0.44 a
	FR266	Montcalm	2.50 a	84 a	326 a	0.46 a
	Montcalm	FR266	2.50 a	50 b	449 a	0.39 b
	Montcalm	Montcalm	4.50 b	78 a	436 a	0.46 a
ANOVA	**Source**	**df**		p value		
	Root (R)	1	0.002	0.019	0.481	0.0019
	Shoot (S)	1	0.002	0.213	0.300	0.004
	R*S	1	0.0113	0.063	0.387	0.053
Middle compacted	FR266	FR266	NA	7 ab	84 ab	0.37 a
	FR266	Montcalm	--	9 a	94 ab	0.37 a
	Montcalm	FR266	--	7 ab	119 a	0.36 a
	Montcalm	Montcalm	--	5 b	40 b	0.37 a
ANOVA	**Source**	**df**		p value		
	Root (R)	1	NA	0.07	0.698	0.75
	Shoot (S)	1	--	0.917	0.177	0.95
	R*S	1	--	0.099	0.087	0.77
Bottom	FR266	FR266	NA	57 a	1204ab	0.36 a
	FR266	Montcalm	--	95 b	1667 a	0.36 a
	Montcalm	FR266	--	76 ab	1568 a	0.34 a
	Montcalm	Montcalm	--	28 c	667 b	0.37 a
ANOVA	**Source**	**df**		p value		
	Root (R)	1	NA	0.018	0.252	0.49
	Shoot (S)	1	--	0.534	0.421	0.33
	R*S	1	--	0.006	0.026	0.07

† Soil layer is defined as top, middle, and bottom. Root traits were measured separately for each layer.

‡ Root rot score is on a scale of 1-7, where 1 is no root rot and 7 is completely rotted (Schneider and Kelly, 2000).

*Values that do not share a letter are significantly different as determined by LSD (0.05). Letters are compared down a column.



a. b.

Figure 1. Photos of containers used in Experiment 1 (a) and Experiment 2 (b). The bulk densities of soil are listed in g/cm^3 for each of the 3 layers of containers used in experiment 2.

Chapter 3: The Relationship among Root Architecture Traits, Plant Growth Habit and Tolerance to Low Soil Phosphorus Levels in an Andean Bean Population

Abstract:

Tolerance to low P soils is a desirable characteristic for common bean line grown in acid-weathered soils of the tropics. Variability for tolerance to low P soils exists in the Andean gene pool. The objective of this research was to identify mechanisms of tolerance to low P soils in a $F_{5:7}$ recombinant inbred line (RIL) population developed from two common bean lines in the Andean gene pool, AND696, an improved line, susceptible to low P soils, with a determinate growth habit and G19833, a landrace tolerant to low P soils, and an indeterminate growth habit. The 77 RILs were grown in low and high soil phosphorus levels in a field in Darien, Colombia. P uptake and root architecture traits were measured in the population, along with P uptake efficiency and seed P content. Genetic differences were identified for root architecture traits, including root length density, specific root length, and average root diameter in the top 16 cm of soil. Specific root length was weakly correlated with P uptake ($r=0.16$) in both the high and low P treatments. P uptake and P use efficiency were correlated with yield. Plant growth habit affected seed yield, and root length density, but was not related to P uptake.

Introduction:

Common bean (*Phaseolus vulgaris* L.) is an important grain legume worldwide, produced on 20 million hectares of land each year. The highest production and consumption of common bean occurs in Latin America (5.6 million metric tons) and Africa (2.8 million metric tons) (FAOSTAT, 2006).

One of the major constraints to bean production in Africa and Latin America is low available soil phosphorus (Wortmann et al., 1998). An estimated 43% of the lands in the tropics are acid weathered soils, specifically Andosols, Ultisoils, and Oxisols, that adsorb or fix P so that 70 to 90% of P fertilizer applied reacts with iron or aluminum to form compounds of low solubility (Sanchez and Salinas, 1981). Common bean is susceptible to low P soils in part due to the high P requirement associated with energy-intensive nodulation and N_2 fixation (Christiansen and Graham, 2002).

Tolerance to low P soils or P efficiency is defined as the ability to produce plant growth and yield in relation to the amount of available phosphorus (Lynch and Beebe, 1995) and can occur by two distinct routes: acquisition efficiency and utilization efficiency. Acquisition efficiency reflects the plants ability to extract P from the environment. It has been shown to be related root system traits that increase the root surface area and allow capture of more P from the soil (Gahoonia and Nielsen, 2003). Utilization efficiency is the superior ability of a plant to convert acquired P into plant biomass and yield and is related to reduced tissue P requirement (Lynch and Beebe, 1995).

Genetic variability for tolerance to low P soils has been identified in common bean (Beebe et al., 1997; Singh et al., 2003). Within the primary gene pool of common bean there are multiple sites of domestication divided into two major groups, Mesoamerican (northern Mexico and Central America) and Andean (South America) (Singh, 2001). The most studied bean lines tolerant to low P soils have been landraces from the Andean gene pool (Lynch and Beebe, 1995; Yan et al., 1996; Bonser et al., 1996; Yan et al., 2004; Beebe et al., 2006).

The Andean landraces identified as tolerant to low P soils have been shown to be efficient in P acquisition from the soil (Liao and Yan, 2001; Liao et al., 2001). Controlled environment physiology studies have identified specific plant root traits related to P uptake in these P efficient lines, including increased lateral and adventitious root number and more shallow basal roots. (Lynch and Brown, 2001; Miller et al., 2003). QTL studies employing inter-gene pool crosses have identified regions of the bean genome important for both root growth and P uptake (Liao et al, 2004; Beebe et al., 2006; Ochoa et al., 2006). Within a recombinant inbred line population developed from a P efficient, Andean bean, G19833 and a P inefficient, Mesoamerican bean DOR364, QTL were identified for total root length and specific root length on two different linkage groups that co-localized with QTL for phosphorus uptake. The uptake QTL and the root QTL each explained about 15% of phenotypic variation for these traits (Beebe et al., 2006).

Low P tolerant, Andean landraces perform well in low P soils, but typically do not yield well in P sufficient soils. They have an indeterminate

growth habit, long maturation time, and are day length sensitive (Beebe et al., 1997). There is question as to whether the features of these landraces that enhance yields in low P environments are the same features that limit their yield potential in other environments. Previous observational studies with common bean support a coordinated growth habit of the root and shoot such that indeterminate plant types with highly branched shoot architecture and little apical dominance also exhibited highly branched root architecture (Lynch and van Beem, 1993). The parental material used in this study had contrasting plant growth habit, and the population was segregating for growth habit, which allowed the opportunity to look at the relationship among growth habit, root architecture, and P acquisition efficiency.

The first objective of this study was to identify the broad mechanisms (P acquisition and P use efficiency) of tolerance to low P soils in a bean recombinant inbred line (RIL) population developed from an Andean intra-gene pool cross between a P efficient, unadapted landrace, G19833 and a P inefficient line, AND696. Specific root architecture traits were also examined for their relationship to P acquisition efficiency. The RIL population was grown in both P deficient and P sufficient soils and plants were evaluated in each environment to clearly identify traits generally important for plant yield vs. those specifically important for adaptation to low P soils.

The second objective of this study was to determine if there were differences in shoot growth habit associated with differences in root growth and if they were related to P uptake in common bean.

Materials and Methods:

Seventy seven $F_{5:7}$ recombinant inbred lines were developed from a cross between two common bean lines from the Andean gene pool. The initial cross G19833 x AND696 was advanced to the F_5 generation by single seed descent and seed of the RILs was increased for field studies. The parent G19833 is a Peruvian landrace with an indeterminate (Type III) growth habit. G19833 has large yellow and red mottled seed with an average 100 seed weight of 41g. G19833 is relatively unadapted (i.e. low yield potential) but has been identified as tolerant to low P soils (Yan et al., 1995ab). The parent AND696 is a CIAT improved line from the race Nueva Granada. It has a determinate growth habit (Type I) and has large red and white mottled seed with an average 100 seed weight of 51g. AND696 has been identified as susceptible to low phosphorus soils (CIAT, 2000).

The RIL population developed from G19833 and AND696 segregated for plant growth habit, 56 of the lines have a determinate growth habit and 21 lines have an indeterminate growth habit. Growth habit in common bean is controlled by a single gene, *fin* (Norton, 1915). The number of determinate and indeterminate lines deviated significantly from a 1:1 ratio that is expected for a single gene trait. This deviation is likely due to the selection of RILs with uniform days to maturity, which biased the selection to favor determinate lines.

In 2000 and 2005, the 77 RILs, G19833, AND696, and two check varieties, G4017 (Carioca) and G16140 were planted in Darien, Colombia in a 9x9 lattice design with three replications at two soil P levels, low P (45 kg/ha triple super phosphate) and high P (300 kg/ha triple superphosphate). Phenotypic

data was collected on 75 of the 77 RILs. The soil of this site in an Andisol with a native soil P of 2 mg/kg based on Bray II extraction method. Seed was hand-planted at 10 cm spacing in 4 row plots. Each row was 3 m in length. The middle two rows were planted to the genotypes of interest and the outer two rows were border rows of Mesoamerican tan seeded cultivar BAT 477. The border rows were included to improve uniformity in soil P availability to the different genotypes and also aided in identification of genetic material.

During the 2005 growing season, various plant measurements were taken to elucidate underlying factors involved in differences between the parental genotypes in tolerance to low soil P. Adventitious root number was counted on twoplants for each P treatment and in each replication at 21 days after planting. This was done by excavating entire root systems from the ground. At mid-pod fill (which ranged from 54 to 67 days after planting) shoots from 50 cm row length (5-10 plants) were harvested and oven dried at 70°C until free of moisture (about 5 days). Dry weights were recorded and shoots were ground to a fine powder in a Wiley mill with a 60-mesh screen. Plant tissue samples were Kjeldahl acid digested and subsequently measured for concentration of P according to the method of Murphy and Riley (1962) using colorimetric spectrometry.

At mid pod fill, a single root sample was taken per plot using a soil auger with a diameter of 7.1 cm and to a depth of 16 cm. Samples were taken directly adjacent to the plant stem. Roots were separated from soil and washed with water. Soil was dried and weighed to determine volume. Cleaned roots were placed in a transparent plastic tray (22 x 28 x 6.5 cm) and covered with water. The tray

containing roots was then scanned with a flat bed scanner with a top and bottom light source at 300 dpi to produce a 2 dimensional scanned image. The images were analyzed with WinRHIZO software (Regent Instruments Inc., Quebec, Canada). Root length, root surface area and average root diameter of the samples was determined. Following image capture, roots were dried at 65°C for five days and dry weights were determined.

Seeds reached maturity at 76 to 98 days after planting. At maturity, seeds were hand-harvested. Seeds were dried and seed yield was determined at 18% moisture. Seed weight (of 100 seed) was measured at 18% moisture for the 2000 and 2005 plantings. A sub sample of seed from the 2000 and 2005 harvest was analyzed for total phosphorus concentration. Five grams of seed of each treatment for each of the three replications were cleaned with distilled water and dried. Samples were then freeze dried to remove all moisture. Freeze-dried seed samples were placed into aluminum tubes containing three silver balls. Mechanical agitation was used to crush seeds into a fine powder. Seed samples were Kjeldahl acid digested and subsequently measured for concentration of P according to the method of Murphy and Riley (1962) using colorimetric spectrometry.

Statistical analysis was conducted with SAS for Windows V8 (SAS Institute, Cary, NC). The command PROC GLM was used to determine treatment, genotype and interaction effects. Data was analyzed as a split plot with soil P level as the whole plot and plant genotype as the split plot. The command PROC CORR was used to determine Pearson correlation coefficients among variables.

Effects of plant growth habit on measured variables were determined by means comparisons of determinate vs. indeterminate growth habit types and differences were established using Tukey's test for significance. Bartlett test for variance homogeneity was conducted for growth habit because there were unequal number of determinate (56) and indeterminate (21) RILs.

Results:

P uptake: Significant differences in P uptake were observed between environments such that mean uptake of the RILs was more than 3 times higher in the high P than the low P environment (Table 1 and Table 2). Genotypic variability was observed for P uptake among the RIL population (Table 1). P uptake by the inefficient parent, AND 696 was below the RIL mean in the high P environment, and similar to the RIL mean in the low P environment (Table 2). Significant genotype x environment (G x E) interactions were observed for this trait (Table 1).

P use efficiency: In addition to P uptake efficiency, P use efficiency also exists as a tolerance mechanism to low P soil. In this study P use efficiency was defined as the amount of seed yield per unit of P uptake by the plant. There was variability for P use efficiency between environments and among genotypes. G x E was not significant (Table 1). Efficient use of P was greater in the low P environment, but genotypic differences in the population were only observed in the high P environment (Table 2).

Seed P content: Another measure of P efficiency is the content of P in the seed. There were significant environmental and genetic differences for seed P content observed, and a G x E effect was present in 2000, but not 2005 (Table 1 and Table 2). The seed P content of AND696 was higher than the mean RIL content in both the high and low P soil treatments (Table 2).

Yield: Significant differences in seed yield were observed between the high and low soil P environments in the RIL population (Table 1). The mean yield in low P was 597 kg/ha and 1345 kg/ha in high P in 2005. Similar yield differences as those seen in 2005 were also recorded between the environments in 2000 (Table 2). The differences in yield in each environment demonstrate the effects of limiting P on plant growth in this population. Yield of the check variety, Carioca was higher than that of the RIL population across environments and years (Table 2). In both years, there was significant genotypic variability for yield and in 2005 there was a significant G x E interaction (Table 1).

Correlations: P uptake was significantly correlated with seed yield in 2005 in each P treatment, and the correlation coefficient was higher in the low P ($r=0.53$) than the high P ($r=0.48$) treatment (Table 3). This is consistent with P uptake having an important role in yield determination in a P limiting environment. P use efficiency was more strongly correlated with seed yield in the high P than the low P treatment (Table 3). There was a negative correlation between P uptake and P use efficiency. This was expected because by definition, P use efficiency is based on getting the highest yields with the least amount of P

uptake. Seed P content was positively correlated with seed yield in 2005, but not in 2000 (Table 9).

Root traits and P uptake: A selection of root traits was measured in the 2005 growing season to determine their importance in P uptake. Adventitious root number was counted at 3 weeks after planting. These roots arise from shoot tissue, and have a lower carbon construction cost than roots arising from root tissue, as they have low density (Lynch and Ho, 2005). They are generally shallow in orientation and deploying in the topsoil therefore closer to P enriched sources of the soil, such as organic matter. Environmental differences were observed for adventitious root number (Table 5). No genotypic differences were detected for adventitious root number in this population (Tables 4 and 5).

Mean squares from analysis of variance identified significant genotypic differences in root length density, specific root length, root surface area, and average root diameter (Table 4). Further inspection of these traits indicated that in the low P soil treatment, only root length density and root surface area were different among the RILs in this study (Table 5). These traits were not however correlated with P uptake. Only specific root length was weakly correlated with P uptake in the low P soil treatment (Table 6).

In the high P soil environment, root length density, root surface area, and average root diameter showed genotypic variation within the RILs (Table 5). In this environment there were also genotypic differences in P uptake (Table 2). There was a weak correlation between specific root length and P uptake and a negative correlation between average root diameter and P uptake (Table 6).

The weak correlations presented in this study between root architecture traits and P uptake are not surprising because roots are extremely plastic, especially in field environments. However, despite the plastic nature of root growth it was possible to detect genetic differences in these traits. The observation that there was genotypic variability for root architecture traits, but these traits were not correlated with P uptake shows that these traits should not be considered adaptive for tolerance to low P soils in this population.

Differences by Growth Habit

In this study, the RIL population segregated for indeterminate and determinate plant growth habit, this trait is controlled by a single gene, the *fin* gene (Koinange et al., 1996; Norton et al., 1915). Plant growth habit has been shown to be linked to maturity in common bean (Coyne and Schuster, 1974; White et al., 1991) as observed here. Determinate RILs in the population reached maturity on average in 80 to 82 days whereas indeterminate RILs required 88 to 89 days to reach maturity depending of soil P level (Table 7).

There were differences in seed yield in the determinate and indeterminate RILs in both 2000 and 2005. The trend however was not the same across years. In 2000, the indeterminate RILs had greater seed yield than the determinate RILs under low P and no differences in yield under the high P treatment (Table 7). Determinate plants have separate vegetative and reproductive stages of development, whereas indeterminate plants continue to grow vegetatively during flowering and seed development (Huyghe, 1998). In 2000, the indeterminate plants yielded higher on average than the determinate plants, perhaps because of

the ability to grow longer and acquire nutrients whereas the determinate plants had switched fully to seed production.

In 2005 there were no differences is seed yield in low P between the determinate and indeterminate plants. Yield was however greater in the determinate plants in the high P treatment (Table 7). Comparison of the 2000 and 2005 yield data suggest the presence of an additional stress present in 2005 that affected the indeterminate RILs to a greater extent than the determinate RILs. Such a stress may have become a factor later in the life cycle of the plants because the indeterminate lines took 6-9 days longer to reach maturity.

In 2005, no significant differences in shoot dry weight or P uptake were observed for determinate as compared to indeterminate RILs (Table 7). There were differences observed in two root traits, but these differences were only present in the high P soil treatment. The indeterminate plants had greater root length density and root surface area than determinate RILs in the population (Figures 1 and 2). Specific root length was weakly correlated with P uptake in the determinate RILs in the low P treatment and with the indeterminate RILs in the high P treatment (Table 8).

The determinate RILs had a higher P use efficiency in high P than the indeterminate RILs. In 2005 the seed P content was higher in the indeterminate RILs in both the high and low P treatments. In 2000 differences in seed P content were only present in the high P treatment (Table 7). Correlations for seed yield and seed P content were significant and positive in 2005 but not in 2000 (Table 9). Since there were unequal number of determinate and indeterminate RILs in the

population, Bartlett's test for variance homogeneity was conducted to identify bias that may have existed in trait analysis by growth habit. Most traits exhibited variance homogeneity although there were exceptions, including seed P content (high P), yield (low P, 2000) and root length density, specific root length, and root surface area (high P) (Appendix, Tables A3-1; A3-2).

Discussion:

Seed yields were greatly reduced in the low P treatment, and genetic variability was observed for response to low soil phosphorus. The population also exhibited genetic differences in P uptake and P use efficiency, although only P uptake efficiency was important in the low P soil treatment. Tolerance to low soil P exhibited by the efficient parent G19833 has been shown to be based on its efficient uptake of P from the soil, which can be attributed to its extensive root system (Liao et al., 2001; Nielson et al., 2001). Root architecture traits that increase the area of soil exploration may in turn increase the capture of immobile P ions by a plant root system (Gahoonia and Nielsen, 2003). Each of the root traits measured in this study are indicators of explorative capacity and we hoped to be able to use the RILs to identify those root traits important for P uptake. None of genetic variability observed for the root architecture traits studied here factored into improved P uptake efficiency.

Previous studies have, however, identified adventitious root number as important for P uptake (Miller et al., 2003) and studies of RILs developed by a cross between an Andean and Mesoamerican line have shown increased numbers of adventitious roots to be important for P uptake (Ochoa et al., 2006). Root

length density and root surface area are both measures of root spacing in a volume of soil. Specific root length and average root diameter indicate root thickness. Thinner roots have an advantage in P uptake over thicker roots because they require less construction cost in the form of carbon, to explore the same area of soil (Lynch and Brown, 2001). Most plant available P is found in the topsoil, therefore in this study, measurements on root traits were conducted on roots in the top 16 cm of soil. Perhaps within the Andean gene pool there is less variability for root traits than in the Mesoamerican gene pool.

Genetic variability was uncovered for P use efficiency in the high P treatment. This trait was associated with the determinate plants (Table 7), and may be indicative of the different growth strategies of indeterminate and determinate plants. In rice (*Oryza sativa* L.), for example, P use efficiency was negatively correlated with plant dry weight and found to be an adaptation to low soil P found in plants inefficient in P uptake (Wissuwa et al., 1998).

Selection of genotypes with greater seed P content may improve plant growth in low P soils. Nutrients stored in the seed are important for germination and early plant growth (Liptay and Arevalo, 2000). Increased seed P content partially compensated for the negative effects of low P soils on early seedling growth in wheat (*Triticum aestivum* L.), by increases in shoot dry weight, leaf size, and root length (De Marco, 1990). A positive correlation between seed P and yield was observed in 2005. The correlation between these traits was stronger for the indeterminate than the determinate lines and therefore may be a more

important factor when specifically selecting indeterminate lines adapted to low soil P.

Another important factor that may be related to plant growth habit is adaptation, because the parents exhibited differences for this trait. The unadapted nature of G19833 can be seen in Figures 3 and 4, where seed yield in high P is regressed by seed yield in low P in 2000 and 2005. G19833 did not perform well in the high P environment (Figure 4). AND696 on the other hand is an improved line and demonstrated an average yield potential in the high P environment, although it performed poorly in the low P environment (Figure 3).

Based on the yield regressions, it would appear that selecting a determinate plant type would be the best way to maximize yield potential across environments. In 2005, the top yielding lines across environments possessed a determinate growth habit (Figure 3), whereas in 2000, the indeterminate RILs on average performed better than the determinate lines, but numerous determinate lines performed well in both environments (Figure 4). In a previous study of Andean beans conducted in three environments in Colombia, determinate lines were found to have greater yield stability (White et al., 1992). Determinate lines are not always more stable and other studies in temperate environments have shown indeterminate lines to be more stable (Kelly et al., 1987).

Conclusions

Genotypic differences were observed for yield in 2000 and 2005. There were also genotypic differences for P uptake in the low P treatment and P uptake

and P use efficiency in the high P treatment. Both P uptake and P use efficiency were correlated with yield. Genetic variability for root length density and root surface area was observed among the RILs. These differences did not appear to improve adaptation to low P soils, however, because they were not correlated with P uptake.

Growth habit affected seed yield differently between years, and was not related to P uptake in 2005. Root length density and root surface area were greater in indeterminate plants in the high P treatment only. Determinate lines were more P use efficient in high P and indeterminate lines had greater seed P content in high and low P in 2005 and in low P in 2000. The differences in trait response in 2000 and 2005 suggest that while the indeterminate lines were (on average) better adapted to the low P environment than the determinate lines, the determinate lines were better able to perform across the two environments.

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Table 1. Analysis of variance of traits related to phosphorus uptake and use, and seed yield in a population of 75 recombinant inbred lines from the AND696/G19833 population field grown in Darien, Colombia in 2000 and 2005 under two treatments: high and low soil phosphorus.

Source	df	Mean squares				
		P uptake	P use eff.	Days to Maturity	Seed P content	Seed yield
				2005		
Genotype (G)	74	263***	0.04**	113***	2092***	161674***
Env. (E)	1	120770***	5.26***	217***	324541***	62929473***
G x E	74	220***	0.02 ns	5.4 ns	420 ns	103183**
				2000		
Genotype (G)	74	-----	-----	55***	1672***	465740***
Env. (E)	1	-----	-----	195***	205516***	88298547***
G x E	74	-----	-----	3.2 ns	673***	211912 ns

ns indicates not significant

*** Indicates significance at $P < 0.05$.**

****Indicates significance at $P < 0.01$.**

***** Indicates significance at P<0.001**

Table 2. Mean plant growth traits in high (HP) and low (LP) phosphorus soils conditions for parents AND696 and G19833 and the means and ranges of 75 recombinant inbred lines (RILs) developed from the parents. Means are also included for the check variety, Carioca. The experiment was planted in 2000 and 2005 in Darien, Colombia. Mean values are of 3 replications. P value indicates level of significant genotypic differences among the RILs for each trait.

		Parents		Recombinant inbred lines			Check
Traits	**P level**	**AND 696**	**G 19833**	**mean**	**range**	**p value**	**Carioca (G4017)**
P uptake ($mg \cdot plant^{-1}$)	**HP**	36.5	92	46.7	25-92	0.0010	70.9
	LP	14.5	-----	13.7	6.6-26.1	0.0112	8.9
P use efficiency ($g\ seed\ mg\ P^{-1}$)	**HP**	0.33	-----	0.29	0.11-0.50	0.0016	0.29
	LP	0.27	-----	0.51	0.25-0.78	0.2787	0.79
Days to maturity 2005	**HP**	77.3	-----	82.9	77-98	<.0001	86.3
	LP	77.3	-----	84.2	77-94	<.0001	84.7
Days to maturity 2000	**HP**	-----	93	83.4	77-90	<.0001	86.7
	LP	-----	93	84.7	80-93	<.0001	83.3
Seed P content ($mg\ 100\ seed^{-1}$) **2005**	**HP**	198	165	193	146-261	<.0001	111
	LP	147	-----	139	97-181	<.0001	87
Seed P content ($mg\ 100\ seed^{-1}$) **2000**	**HP**	-----	161	160	107-256	<.0001	93
	LP	-----	-----	116	76-151	<.0001	68
Seed yield ($kg \cdot ha^{-1}$) **2005**	**HP**	1347	-----	1345	582-1962	<.0001	2096
	LP	465	-----	597	323-944	0.0542	830
Seed yield ($kg \cdot ha^{-1}$) **2000**	**HP**	-----	1080	1516	681-2871	<.0001	3038
	LP	-----	649	642	268-1064	<.0001	1344

Table 3. Phenotypic correlations among P uptake, P use efficiency, and seed yield in a population of 75 recombinant inbred lines from a AND696/G19833 cross grown in high (HP) or low (LP) soil phosphorus in Darien, Colombia in 2005.

	P level	P use efficiency	Seed yield
P uptake	HP	-0.51***	0.48***
	LP	-0.59***	0.53***
P use efficiency	HP	------	0.43***
	LP	------	0.24***

***Indicates significance at $P < 0.05$.**
****Indicates significance at $P < 0.01$.**
***** Indicates significance at P<0.001.**

Table 4. Analysis of variance of root traits in a population of 75 recombinant inbred lines from the AND696/G19833 population field grown in Darien, Colombia in 2005 in two environments: high and low soil phosphorus.

Source	df	Mean Squares				
		Adv. roots	Root length density	Specific root length	Root surface area	Average root diameter
Genotype (G)	74	5^{ns}	0.56^{***}	21^{*}	2210^{***}	0.008^{***}
Environment (E)	1	142^{***}	9.06^{***}	31^{ns}	691^{ns}	0.065^{***}
G x E	74	7^{ns}	0.31^{ns}	13^{ns}	1538^{ns}	0.006^{ns}

^{ns} indicates not significant
*** Indicates significance at $P < 0.05$.**
****Indicates significance at $P < 0.01$.**
***** Indicates significance at P<0.001**

Table 5. Mean root growth traits in high (HP) and low (LP) phosphorus soils conditions for parents AND696 and G19833 and the means and ranges of 75 recombinant inbred lines (RILs) developed from the parents. Means are also included for the check variety, Carioca. The traits are from the 2005 field experiment planted in Darien, Colombia. Mean values are of 3 replications. P value indicates level of significant genotypic differences among the RILs for each trait.

		Parents		Recombinant inbred lines			Check
Traits	**P level**	**AND 696**	**G 19833**	**mean**	**range**	**p value**	**Carioca (G4017)**
Adventitious roots (#)	**HP**	7.2	8.5	9.9	7-12	0.8931	8.2
	LP	11.2	-----	8.8	6-14	0.5291	9.2
Root length density ($cm \cdot cm^3$-1)	**HP**	1.35	1.1	1.08	0.57-2.30	0.0008	0.70
	LP	1.59	-----	1.36	0.59-2.59	0.0281	0.66
Specific root length ($cm \cdot mg^{-1}$)	**HP**	11.1	10.7	8.4	3.3-17.6	0.5144	5.7
	LP	9.71	-----	8.8	4.0-18.7	0.3887	4.4
Root surface area (cm^2)	**HP**	87.9	57.3	71.7	33-147.4	0.0040	53.4
	LP	60.3	-----	74.0	32.2-156.5	0.0289	37.7
Average root diameter (mm)	**HP**	0.24	0.27	0.33	0.25-0.53	0.0128	0.37
	LP	0.26	-----	0.31	0.24-0.40	0.3464	0.32

Table 6. Phenotypic correlations between root traits and P uptake and seed yield in a population of 75 recombinant inbred lines developed from a AND696/G19833 cross grown in high (HP) or low (LP) soil phosphorus in Darien, Colombia in 2005.

	P level	Adv. root number	Root length density	Specific root length	Root surface area	Average root diameter
P uptake	HP	-0.04	-0.06	0.16**	-0.16**	-0.18***
	LP	-0.08	0.001	0.16**	0.002	-0.01
Seed yield	HP	0.09	-0.098	-0.04	-0.10	-0.05
	LP	-0.099	0.03	0.10	0.02	-0.022

***Indicates significance at $P < 0.05$.**
****Indicates significance at $P < 0.01$.**
***** Indicates significance at P<0.001.**

Table 7. Means of root and shoot traits of 75 RILs developed from a AND696/G19833 cross and grown under high and low soil phosphorus treatments in Darien, Colombia in 2000 and 2005, grouped and averaged by plant growth habit of which there were two categories, indeterminate (Ind.) and determinate (Det.).

Traits	High P		Low P	
	Ind.	**Det.**	**Ind.**	**Det.**
		2005		
Adventitious roots (#)	10.2 *a*†	9.8 *a*	8.7 *a*	8.9 *a*
Root length density ($cm \cdot cm^3$-1)	1.26 *a*	1.01 *b*	1.42 *a*	1.34 *a*
Specific root length ($cm \cdot mg^{-1}$)	9.0 *a*	8.2 *a*	8.8 *a*	8.9 *a*
Root surface area (cm^2)	85.0 *a*	63.5 *b*	76.9 *a*	71.3 *a*
Average root diameter (mm)	0.34 *a*	0.32 *a*	0.31 *a*	0.30 *a*
Shoot dry weight (g ·plant^{-1})	14.2 *a*	13.7 *a*	6.6 *a*	6.3 *a*
P uptake (mg ·plant^{-1})	46.4 *a*	46.6 *a*	13.7 *a*	13.7 *a*
P use efficiency (g·seed mg P^{-1})	0.25 *a*	0.30 *b*	0.47 *a*	0.52 *a*
Days to maturity	88.6 *a*	80.6 *b*	89.4 *a*	82.2 *b*
Seed P content (mg 100 seed^{-1})	210 *a*	186 *b*	147 *a*	136 *b*
Seed yield ($kg \cdot ha^{-1}$)	1138 *a*	1435 *b*	573 *a*	605 *a*
		2000		
Days to maturity	86.7 *a*	82 *b*	88.3 *a*	83.3 *b*
Seed P content (mg 100 seed^{-1})	176 *a*	153 *b*	112 *a*	117 *a*
Seed yield ($kg \cdot ha^{-1}$)	1436 *a*	1551 *a*	707 *a*	614 *b*

† Significant differences are based on Tukey tests and are at alpha = 0.05. Values that do not share the same letter are significantly different. Tests should be read across rows and within each phosphorus treatment level, High P or Low P.

Table 8. Phenotypic correlations between root traits and P uptake and seed yield in a population of 75 recombinant inbred lines (RILS) developed from a AND696/G19833 cross grown under high or low soil phosphorus treatments in Darien, Colombia in 2005. For the analysis, RILs were grouped according to plant growth habit of which there were two categories, indeterminate (Ind.) and determinate (Det.).

	Growth habit	**Adv. root #**	**Root length density**	**Specific root length**	**Root surface area**	**Average root diameter**
				Low P		
P uptake	Det.	-0.10	0.02	0.20***	0.02	-0.04
	Indet.	-0.01	-0.03	-0.08	-0.03	0.05
Seed yield	Det.	-0.04	0.13	0.17**	-0.10	0.10
	Indet.	-0.27***	-0.17	-0.05	-0.15	0.19
				High P		
P uptake	Det.	-0.09	-0.12	0.10	-0.17**	-0.14*
	Indet.	0.04	0.03	0.24***	-0.13	-0.27***
Seed yield	Det.	0.15*	0.10	0.14*	0.05	-0.04
	Indet.	0.04	-0.18	0.02	-0.13	-0.005

*** Indicates significance at $P < 0.05$.**
****Indicates significance at $P < 0.01$.**
***** Indicates significance at P<0.001**

Table 9. Phenotypic correlations between P uptake and seed yield and P seed content and seed yield in a population of 75 recombinant inbred lines (RILs) from a AND696/G19833 cross grown under high or low soil phosphorus treatments in Darien, Colombia in 2000 and 2005. For the analysis, RILs were grouped according to plant growth habit of which there were two categories, indeterminate (Ind.) and determinate (Det.).

	High P-Ind.	High P-Det.	Low P-Ind.	Low P-Det
Seed yield	P uptake (2005)			
	0.03	0.18**	0.46***	0.42***
	P seed content			
	2005			
	0.17	0.30***	0.42***	0.30***
	2000			
	-0.06	0.12	0.08	0.08

*** Indicates significance at $P < 0.05$.**
****Indicates significance at $P < 0.01$.**
***** Indicates significance at P<0.001**

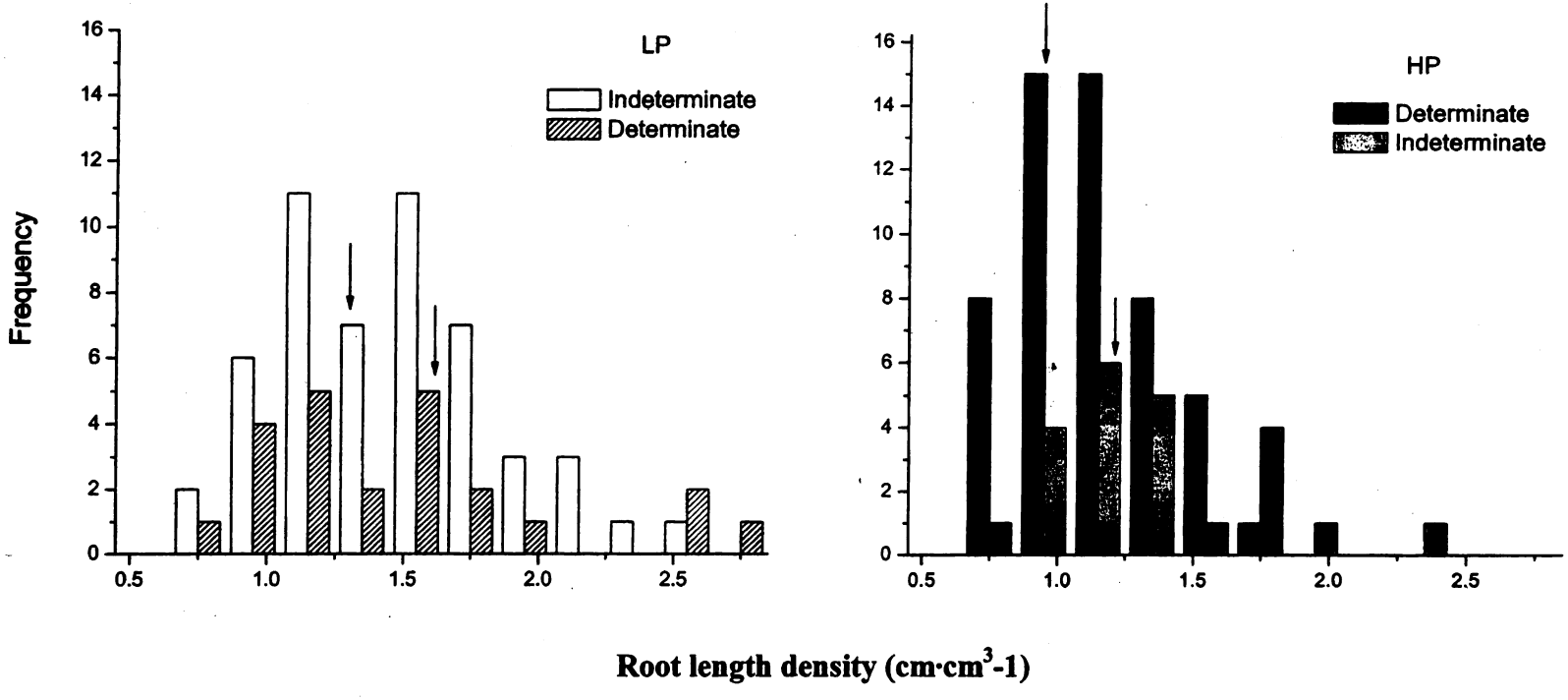
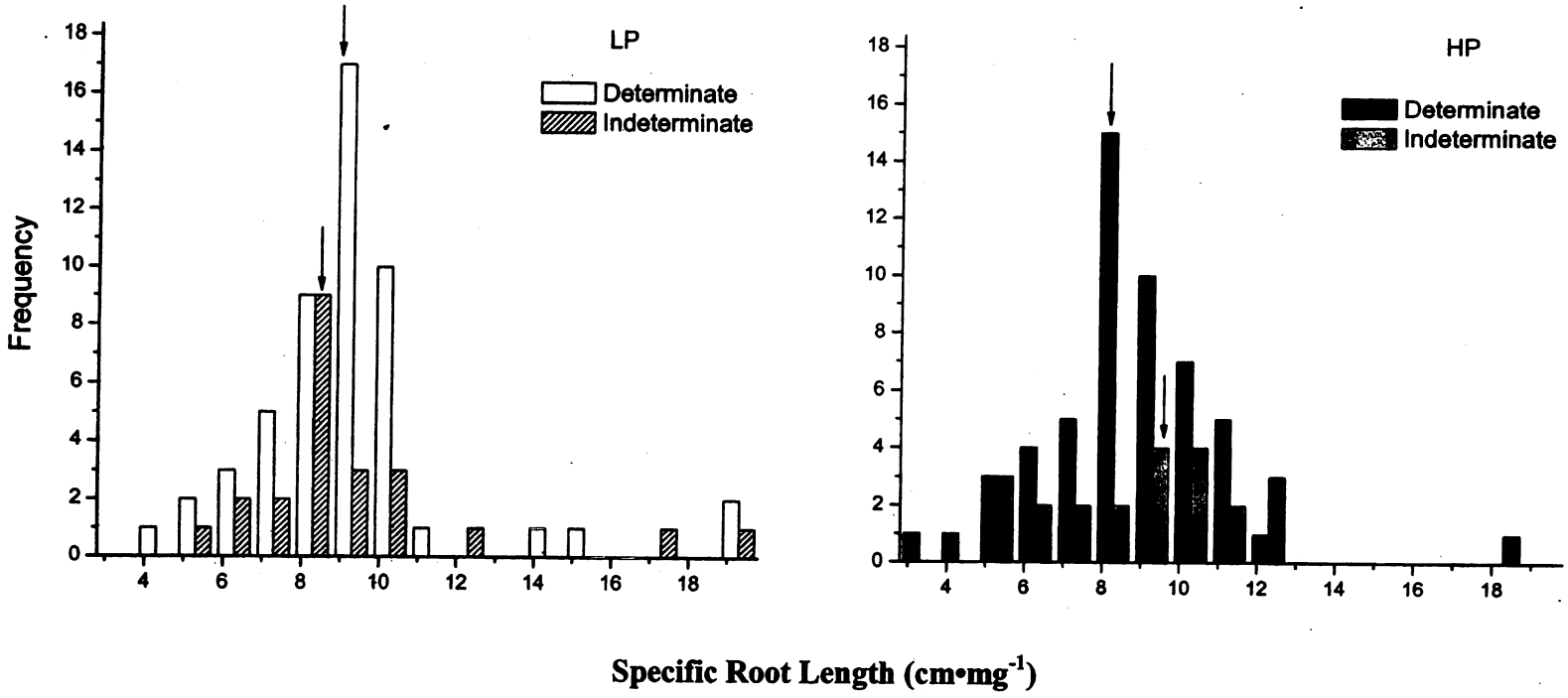


Figure 1. Frequency distribution of root length density and specific root length in 75 recombinant inbred lines (RILs) developed from a cross between AND696 and G19833 planted in Darien, Colombia in 2005 in high (HP) and low (LP) phosphorus soil. The distribution of RILs in LP is shown on the graphs on the left and on graphs on the right show distribution under HP. Frequency distributions are separated by soil P level with LP represented by graphs on the left and HP by graphs on the right. Within each graph RILs are also separated by growth habit. Arrows represent mean values of determinate and indeterminate RILs within each treatment. Means are the average of 3 replications.

Figure 1 (cont'd).



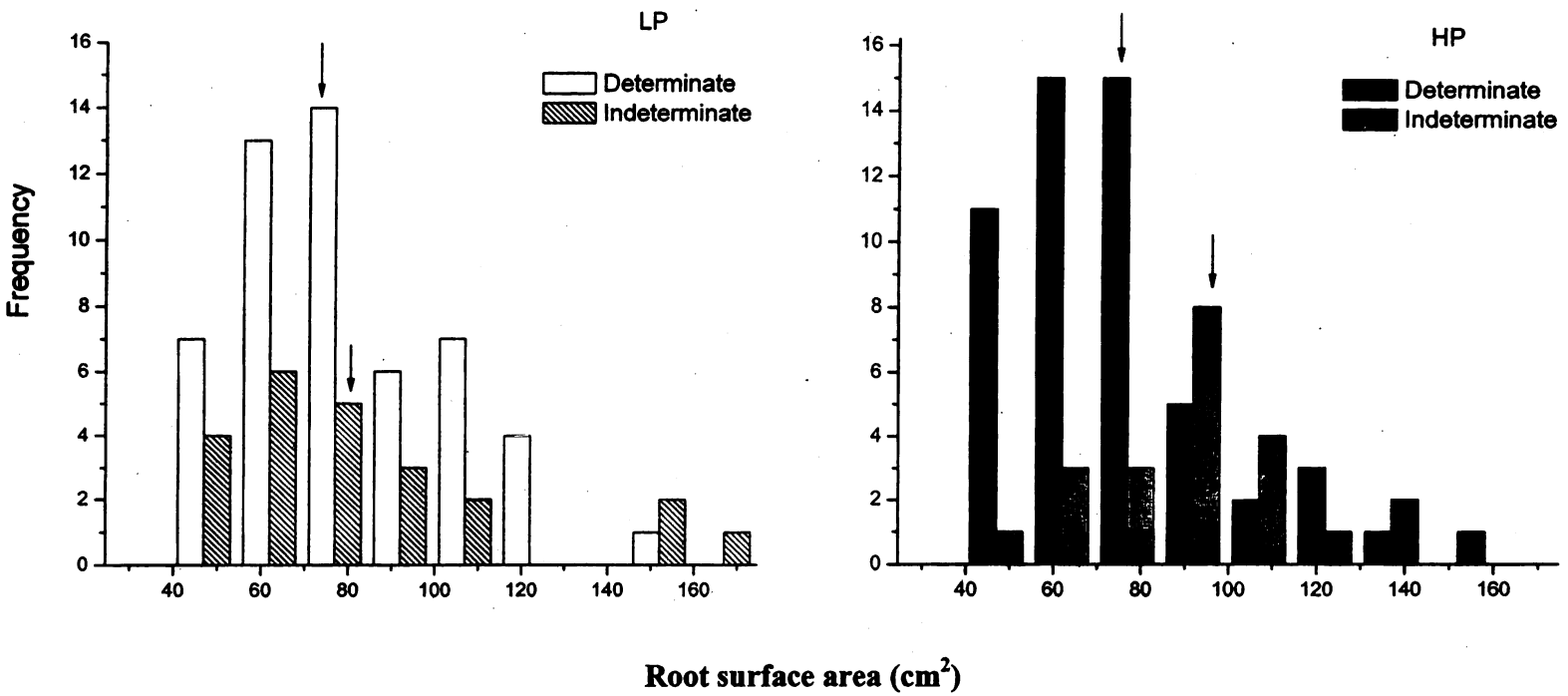
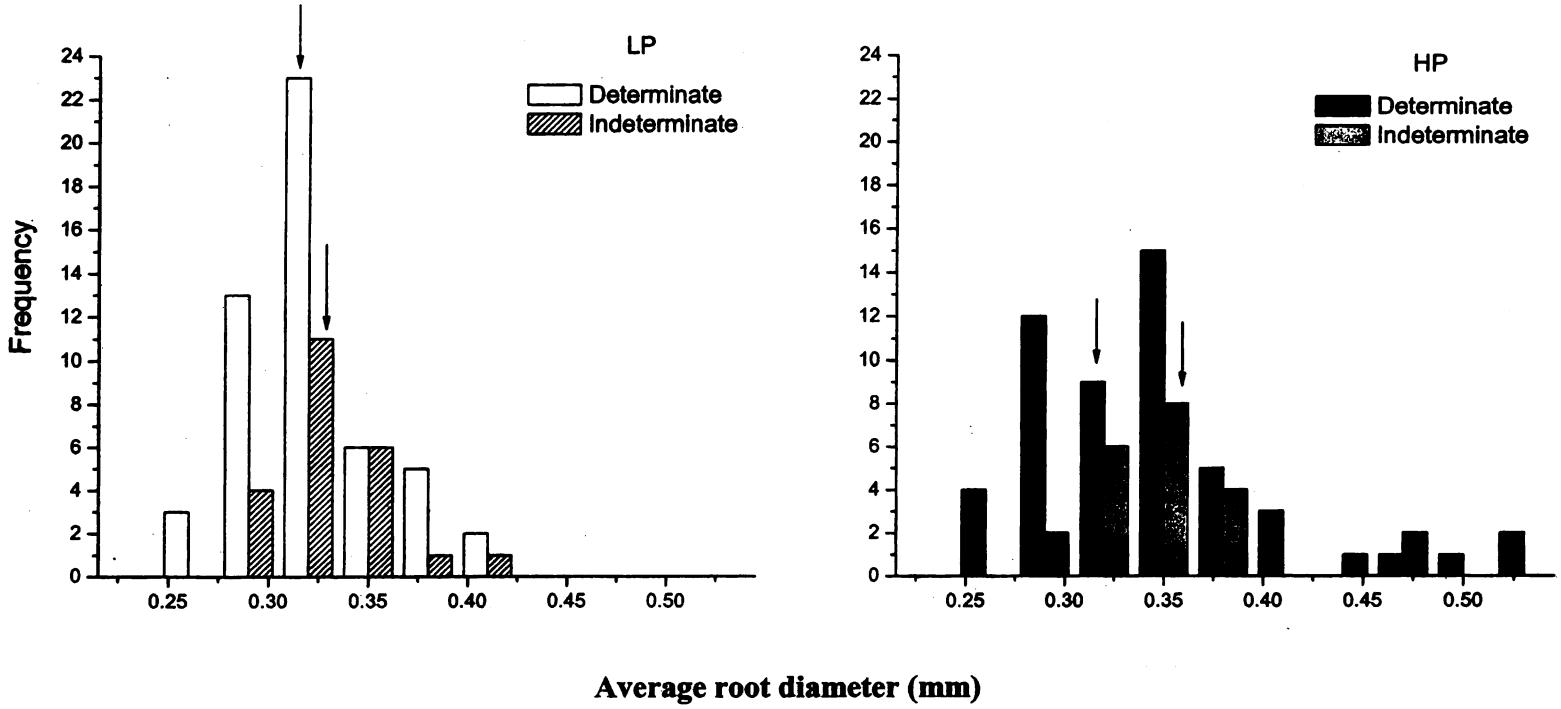


Figure 2. Frequency distribution of root surface area and average root diameter in 75 recombinant inbred lines (RILs) developed from a cross between AND696 and G19833 planted in Darien, Colombia in 2005 in high (HP) and low (LP) phosphorus soil. The distribution of RILs in LP is shown on the graphs on the left and on graphs on the right show distribution under HP. Frequency distributions are separated by soil P level with LP represented by graphs on the left and HP by graphs on the right. Within each graph RILs are also separated by growth habit. Arrows represent mean values of determinate and indeterminate RILs within each treatment. Means are the average of 3 replications.

Figure 2 (cont'd).



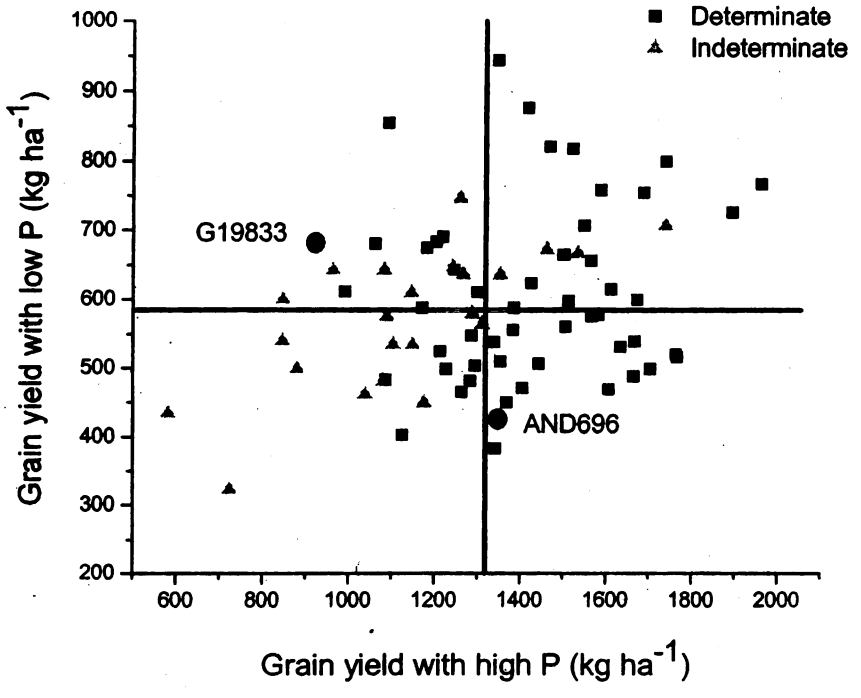


Figure 3. Regression of seed yield under low P by seed yield under high P in the 2005 growing season of the population in 75 recombinant inbred lines developed from a cross between AND696 and G19833. Horizontal bar represents mean yield under low P and vertical bar represents mean yield under high P. Note: G19833 yield values are shown from 2000, not 2005, because seed of this parent was not planted in 2005.

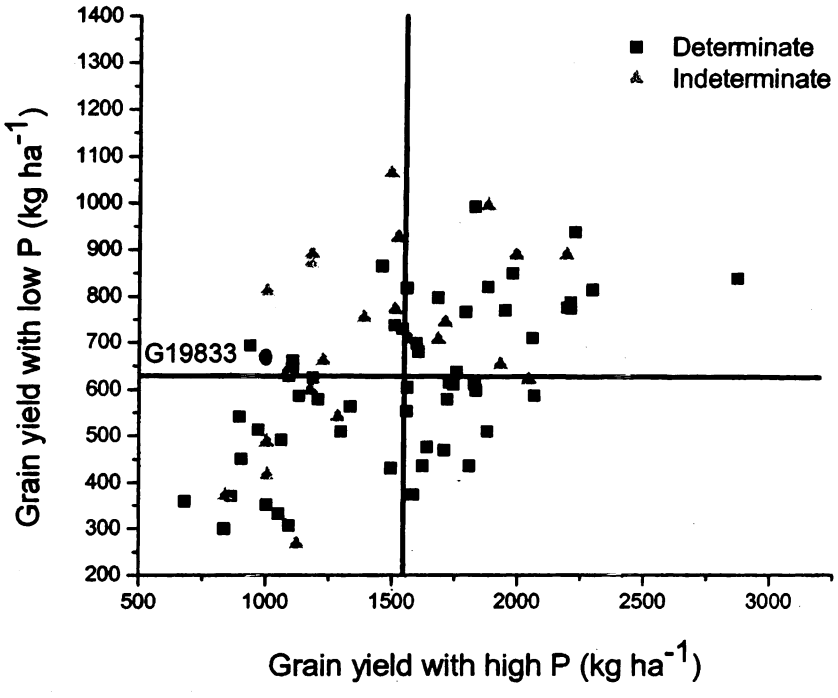


Figure 4. Regression of seed yield under low P by seed yield under high P in the 2000 growing season of the population in 75 recombinant inbred lines developed from a cross between AND696 and G19833. Horizontal bar represents mean yield under low P and vertical bar represents mean yield under high P.

Chapter 4: Identification of QTL related to Root Architecture Traits and Low Phosphorus Tolerance in an Andean Bean Population

Abstract

Low soil phosphorus is a major constraint to common bean production. The identification of QTL (quantitative trait loci) for phenotypic traits associated with tolerance to low phosphorus soils will aid in the development of cultivars that can perform well in such environments. Root architecture was identified as a determinate of P uptake in a cross between an Andean tolerant and Mesoamerican susceptible bean genotype. This experiment was conducted to determine if root architecture aids in tolerance to low P soils in Andean intra gene pool cross between a tolerant (G19833) and susceptible (AND696) genotype. A linkage map was developed from 77 $F_{5:7}$ recombinant inbred lines of G19833/AND696 using SSR, AFLP and RADP markers with 12 linkage groups for a total of 633cM. QTL were identified for root length density (cm/cm^3) and root surface area in the top 16 cm of soil. The QTL were derived from G19833, but did not co-localize with QTL for P uptake. A QTL for P use efficiency, derived from G19833 was identified on linkage group B11. QTL were identified for seed yield on linkage group B1 near the *fin* gene for determinacy, while G19833 was the source of the increased effect in the low P environment in 2000, AND696 was responsible for the yield increase in this location in the high P environment in 2005.

Introduction

Quantitative trait loci (QTL) analysis is a powerful tool for understanding genetic variation and control of complex traits. QTL are regions of the genome statistically associated with phenotypic variation of a quantitative trait (Doerge, 2002). QTL identification requires phenotypic data for a trait of interest in a segregating population and a genetic linkage map (Collard et al., 2005).

Mapping of QTL permits the identification QTL controlling multiple traits in numerous genetic backgrounds. This technique also can be used to dissect epistatic interactions and predict genotypic by environment interactions at the genetic level (Dekkers and Hospital, 2002). The identification of QTL serves as a starting point for marker assisted selection (MAS), which offers potential to improve traits with low heritabilities or difficult to measure traits without a heavy reliance on phenotypic selection (Collard et al., 2005). QTL mapping has also been successfully employed as a first step to identify genes underlying phenotypic variation for a trait of interest via positional cloning (Li et al., 2006).

In common bean (*Phaseolus vulgaris L.*) QTL studies have been conducted for the major diseases, insects, and abiotic stress that limit productivity. To date, QTL analysis for disease resistance has been the most fruitful area of research, and MAS based on QTL studies for bean golden mosaic virus and common bacterial blight resistance are currently employed in breeding programs (Miklas et al., 2006).

QTL analysis for abiotic stresses affecting common bean has yet to reach the level of success of that seen with biotic stress, nonetheless, this tool is

currently being used to identify underlying mechanisms of tolerance to low P soils (Yan et al., 2004; Beebe et al., 2006).

Phosphorus deficiency is one of the most prevalent stresses, especially in bean growing regions of the tropics. An estimated 60% of bean production in Latin America and Africa occurs under deficient soil P conditions (Wortman et al., 1998).

Tolerance to P deficiency has been identified in bean lines from diverse genetic backgrounds in both the Andean and Mesoamerican gene pools (Beebe et al., 1997). A number of Peruvian landraces, including G19839 and G19833, have been found to grow especially well in low P soils (Yan et al., 1995a; 1995b). Strong genotype x environment interactions for seed yield, however, necessitate the use of other indicators of tolerance to low P soils that exhibit higher heritabilities (Beebe et al., 1997). A number of physiological traits correlated with tolerance to low P soils have been identified. In G19833, research has shown P efficiency to be related to greater P uptake from the soil. Root system phenotypes that exhibit shallower basal root angle, greater total root length and root surface area, and root length of basal roots in top 3 cm have been shown to enhance P uptake in P limiting environments (Bonser et al., 1996; Liao et al., 2004; Beebe et al., 2006).

The measurement of root traits in the field is laborious and root growth is very plastic even with small changes in the soil environment (Snapp et al., 1995), making selection for plants with desired root traits challenging. The identification of QTL for root traits that improve P uptake in low P soils is a first

step to conducting MAS for P efficiency. Studies (Beebe et al., 2006 and Liao et al., 2004) using G19833 as the efficient parent in crosses with DOR364, a Mesoamerican small seeded black bean as the inefficient parent, have identified QTL for root traits that co-localize with QTL for P uptake efficiency. QTL for both root length and P uptake in low P soil were found in the same region of linkage group B4 with r^2 values of 0.21 and 0.13, respectively. Additional QTL for specific root length and P uptake under the same environmental conditions co-localized to a region of linkage group B10 and had r^2 values of 0.19 and 0.14, respectively (Beebe et al., 2006). Liao et al. (2004) also identified QTL on linkage group B4 in the G19833/DOR364 population for percent of basal roots in the top 3 cm in a growth pouch assay that co-localized with QTL for P uptake in the field.

The identification of QTL for root architecture traits with QTL for P uptake efficiency in crosses between the Andean and Mesoamerica gene pools raises the question if similar mechanisms for P efficiency would be observed in an Andean x Andean cross using the same P efficient parent, G19833. The first objective of this study was to construct a linkage map of two Andean common bean lines with different tolerance to low P soils. The second objective was to identify QTL related to tolerance to low P soils using an Andean common bean recombinant inbred line population.

Materials and Methods:

Plant Material

The common bean genotypes G19833 and AND696 from the Andean gene pool were used as parents to develop 77 $F_{5:7}$ recombinant inbred lines (RILs). The initial cross was advanced to the F_5 generation by single seed descent. G19833 is a Peruvian landrace with an indeterminate growth habit and large yellow and red mottled seed with an average 100 seed weight of 41g. G19833 has also been identified as tolerant to low P soils (Yan et al., 1995ab). AND696 is a CIAT improved line from the race Nueva Granada with a determinate growth habit and large red and white mottled seed with an average 100 seed weight of 51g. AND696 has been identified as susceptible to low phosphorus soils (CIAT, 2000). The RILs developed from G19833 and AND696 segregated for determinacy, with 56 determinate and 21 indeterminate lines. Growth habit in common bean is controlled by a single gene, *fin* (Norton, 1915). The number of determinate and indeterminate lines deviated significantly from a 1:1 ratio that is expected for a single gene trait. This deviation is likely due to the selection of RILs with uniform days to maturity, which biased the selection to favor determinate lines.

Field Trials

In 2000 and 2005, the 77 RILs, G19833, AND696, and two check varieties, G4017 (Carioca) and G16140 were planted in Darien, Colombia in a 9x9 lattice design with three replications at two soil P levels, low P (45 kg/ha triple super phosphate) and high P (300 kg/ha triple superphosphate). Phenotypic

data was collected on 75 of the 77 RILs. The soil of this site in an Andisol with a native soil P of 2 mg/kg based on bray II extraction method. Seed was hand-planted in 4 row plots where each row was 3 m long. During the 2005 growing season, various plant measurements were taken to elucidate underlying factors involved in the parental genotypes differences in tolerance to low soil P. Plant measurements include adventitious root number at 3 weeks after planting, shoot dry weight and total P concentration at mid pod (which ranged from 54 to 67 days after planting), root length and weight to a depth of 16 cm below ground at mid pod fill (Chapter 3, Materials and Methods).

Seeds reached maturity at 76 to 98 days after planting, as determined by seed dryness. At maturity, seeds were hand-harvested. Seeds were dried and seed yield was determined at 18% moisture. Seed weight of 100 seed was measured at 18% moisture for the 2000 and 2005 plantings. A sub sample of seed from the 2000 and 2005 harvest was acid digested and analyzed for total P concentration according to the method of Murphy and Riley via colorimetric spectrometry (1962).

DNA Isolation and Molecular Marker Analysis

Plant tissue was harvested from 5 plants of greenhouse grown seed of G19833, AND696 and 77 $F_{5:7}$ recombinant inbred lines. Total DNA was extracted from plant tissue with 24:1 Chloroform: Isoamyl alcohol. The miniprep procedure was carried out according to the method of Edwards et al. (1991). DNA was quantified with a fluorometer (Hoefer DyNA Quant 200, San Francisco, CA). Molecular markers screened in the study for polymorphisms between the

parents G19833 and AND696 included 125 simple sequence repeats (SSR) (Metais et al., 2002; Blair et al., 2003; Gaitan-Solis et al., 2002; Yu et al., 2000; Caixeta et al., 2005), 50 Random amplified polymorphic DNA (RAPD) (Integrated DNA Technologies, Inc., Coralville, IA) and 41 amplified fragment length polymorphisms (AFLP) primer combinations.

SSR Markers

Amplification was performed with 2 μl of DNA diluted to 20 ng•μl^{-1}, 0.2 μl of primer, 0.15μl of Taq polymerase, 1.2μl (2.5mM) $MgCl_2$, 1.2μl (10x) PCR buffer, and 0.12μl of a 10mM mix of dNTPs. PCR was conducted in a 96 well PTC-100 Programmable Thermal Controller (MJ Research, Inc., Waltham, MA). The thermocycler was programmed for 1 cycle of 5 minutes at 94°C, followed by 30 cycle of 1 minute at 94°C, 1 minute at 47°C, and 1 minute at 72°C, and a final extension step at 72°C for 5 minutes. Double stranded DNA was denatured for 5 minutes at 94°C. PCR amplification products were separated on 4% polyacryilimide gels and DNA bands were visualized with silver nitrate according to the procedure of Blair et al. (2003).

RAPD markers

Amplification was performed with 5 μl of DNA diluted to 10 ng•μl^{-1}, 1 μl of primer (Integrated DNA Technologies, Inc., Coralville, IA), 0.3μl pf Taq polymerase, 25μl (2.5mM) $MgCl_2$, 25μl (10x) PCR buffer, and 0.5μl of a 10mM mix of dNTPs. PCR was conducted in a 96 well PTC-100 Programmable Thermal Controller (MJ Research, Inc., Waltham, MA). The thermocycler was programmed for 2 cycles of 1 minute at 91°C, 15 seconds at 42°C, and 1 minute

and 10 seconds at 72°C, followed by 38 cycles of 15 seconds at 91°C, 15 seconds at 42°C, and 1 minute and 10 seconds at 72°C, and a final extension step at 72°C for 5 minutes. A total of 20 µl of PCR product were loaded onto1.5% agarose (diluted with 0.5x TBE buffer) gels with ethidium bromide and ran in electrophoresis chambers with 1.5L 0.5x TBE buffer for 3 hours at 110 volts. PCR amplification products were visualized under UV light. RAPD markers were named according to the primer used and the molecular weight of the band in kilobases.

AFLP Markers

AFLP reactions were performed according to the procedure described by Vos et al. (1995), using a commercially available kit (AFLP analysis System I, Invitrogen Corporation, Carlsbad, California) and following the manufacturer's instructions. The digestion of 500 ng of DNA was performed with 2 µl EcoRI/MseI (1.25U/µl), incubated for two hours at 37°C. The restriction fragments were ligated to enzyme adapters using 1µl of T4 DNA ligase (1 U/ µl) incubated for two hours at 20°C. The preamplification (primer +1 base) was performed with 2.5 µl of DNA diluted 1:10 from the digestion-ligation product, 20 µl preamplification primer mix, 2.5 10x PCR plus Mg and 0.15 µl of DNA polymerase was added. PCR conditions were 20 cycles of 94°C for 30 seconds, 56°C for 60 seconds, and 72°C for 60 seconds. The PCR product was run in 1% agarose gel and diluted 1:50. The amplification (primer +3 bases) was performed with 5 µl of DNA's dilution 1:50 and 5µl of Mix I (primers and dNTP's) and 10 µl Mix II (10x buffer, MgCl and DNA polymerase). The PCR conditions were one

cycle at 94°C for 30 seconds, 65°C for 60 seconds, and 72°C for 60 seconds, then the annealing temperature was lowered 0.7°C per cycle for 13 cycles and 23 cycles at 94°C for 30 seconds, 56°C for 60 seconds, and 72°C for 60 seconds. PCR amplification products were separated on 4% polyacryilimide gels and DNA bands were visualized with silver nitrate according to the procedure of Blair et al. (2003). The AFLPs were named by combining the last 2 selective bases ligated to EcoR1 with the last two selective bases ligated to Mse1 in the amplification step.

Genotypic Data Analysis

Linkage analysis was conducted with the software JoinMap 3.0 for Windows (Van Ooijen and Voorrips, 2002) set to Kosambi's map function. Kosambi's mapping function assumes the existence of interference that is negatively related to recombination frequency. Analysis was conducted with marker data of 160 molecular markers segregating in the population of 77 recombinant inbred lines. Mapping parameters were set to recombination frequency smaller than 0.300 and a LOD score larger than 3.0. Parameters were relaxed to a recombination frequency of smaller than 0.45 and a LOD score greater than 2.0 to join unlinked markers and to join fragmented linkage groups. LOD (log of odds) scores are = log (L_1/L_0), where L_1 is the likelihood for the alternative hypothesis and L_0 is the likelihood of the null hypothesis. A LOD score of 3 means the alternative hypothesis is 1000 times more likely than the null hypothesis. Linkage groups were identified according to location of SSR markers (Blair et al., 2003) and were named according to the core bean linkage map (Freyre et al., 1998).

QTL analysis was conducted with the genetic map developed with the JoinMap program and with the phenotypic means for each RIL collected from the field study. The computer software program Windows QTL cartographer version 2.5 (Wang et al., 2006) was used to identify QTL for root length density, root surface area, shoot P concentration, P uptake, seed yield and seed P content. The Composite Interval Mapping (CIM) feature set to a window size of 10cM and with forward and backward regression model was used to identify QTL. CIM involves the use of maximum likelihood estimates and linear regression to identify QTL within marker intervals. Significant QTL were considered by defining the LOD score at p=0.01 after 1000 permutation tests.

Results and Discussion:

Linkage Map

DNA polymorphism levels with the molecular markers used in this study were low to moderate between the parent lines G19833 and AND696. Of the 125 SSR markers screened, all of which were developed for use in common bean, only 26.4% were polymorphic between the parent lines. Low polymorphism levels are often seen in intra gene pool crosses such as this one between two Andean genotypes. A recent study of SSR polymorphism levels compared 129 markers in 44 common bean genotypes from both Andean and Mesoamerican gene pools, and in the study, the average inter gene pool polymorphism level (Andean x Mesoamerican) was 59.6% and intra gene pool polymorphisms were 37.9% (Blair et al., 2006).

The 103 locus linkage map developed from the SSR, RAPD, and AFLP markers screened in the G19833/AND696 RIL population spaned 633 cM. Common bean has 11 linkage groups corresponding to the genome's 11 chromosomes. In this study, 12 linkage groups were identified, 10 of which were able to be identified and named according to the bean consensus map, based on the placement of SSR markers (Freyre et al., 1998; Blair et al., 2003) (Figure 1). Linkage group B5 is missing from the map, although the identities of Groups A and D, (which are comprised solely of AFLP markers), have yet to be determined in relation to the consensus map.

QTL identification

Using composite interval mapping, 21 QTL were identified for 8 traits in 12 marker intervals on 7 linkage groups. One-third of the QTL were clustered on linkage groups B1 and affected more than one trait. An additional 19% of the QTL were located on linkage group B6 and also affected multiple traits. Individual QTL explained 12 to 45% of the phenotypic variation, and total phenotypic variation explained for any one trait was 14 to 60% (Table 1).

Root Architecture

Frequency distribution graphs for four root traits measured at mid pod fill are shown in Figure 2. Means of RILs and parental genotypes for each trait are available in Chapter 3, Table 4. The mean root length density of the RILs was greater in the low P environment (1.36 cm/cm^3) than in the high P environment (1.08 cm/cm^3). QTL identified for root length density in the high P environment were distinct from the QTL identified for the same trait in the low P environment

(Table 1, Figure 6). There were no significant differences for root surface area based on P treatment. However, QTL for this trait were only detected for the high P environment. The QTL found for root surface area in high P mapped to the same linkage groups as the QTL for root length density in high P. The increased effect of each of the root traits was derived from G19833 (Table 1). A QTL for specific root length in greenhouse grown RILs from the population G19833/DOR364 was identified in the same region of linkage group B1 (Beebe et al., 2006) as QTL for root length density and root surface area under high P described here. Additional QTL for root traits in the G19833/DOR364 population were identified in the same region of B3 as the QTL for root length density in low soil P identified in this study. They include a QTL for taproot length in greenhouse grown RILs (Beebe et al., 2006) and a QTL for taproot root hair length in solution culture grown plants (Yan et al., 2004). None of these QTL, however, overlap with QTL for P uptake.

P uptake

Shoot P concentration and P uptake distribution among the RILs was affected by the P level of the soil (Figure 3; Chapter 3, Table 2). The range of values for P uptake was greater in the high P than the low P soil (Figure 3), suggesting luxury consumption of P occurred in some RILs in the high P treatment. QTL were detected on B8 for shoot P concentration and P uptake in the low P environment (Table 1; Figure 6). A QTL for P uptake in the high P environment was found on B11. The increased effect of each of these traits was conferred by AND696. No QTL for P uptake were identified with the 2005 field

data where G19833 increased P uptake. This result is contrary to results found with the G19833/DOR364 population where QTL for p uptake from G19833 were identified (Beebe et al., 2006; Yan et al., 2004). This result, however, may be specific to the 2005 field season, as suggested by the 2000 field season data where a QTL was identified for yield in the low P environment derived from G19833 (Table 1).

Seed Yield

Frequency distribution graphs for seed yield in 2005 and 2000 show the large variability for yield across RILs and soil P levels (Figure 4, bottom; Chapter 3, Table 2). In the low P treatment in 2005, AND696 yielded less than the mean of the RILs (465 and 597 $kg \cdot ha^{-1}$, respectively). In the high P treatment in 2005, AND696 had the same yield as the mean of the RILs (1347 $kg \cdot ha^{-1}$). One QTL was detected for seed yield in 2005 which explained 27% of the phenotypic variation. It was observed only in the high P treatment, and AND696 conferred the increased effect on yield (Table 1). In 2000, one QTL was found for yield in low P and one was found for yield in high P, explaining 17 and 45% of the phenotypic variation, respectively. Each QTL was derived from G19833 (Table 1). The QTL identified for yield under the high P treatment in 2005 and that for yield under the low P treatment in 2000 both map to the same region of B1 and the increased effects on yield for these QTL were derived from different parents (Table 1; Figure 6). The region of B1 where these QTL are located is the same region where the single gene (*fin*) responsible for determinacy is located (Koinange et al., 1996), as the AND696/G19833 population segregated for

determinacy. The efficient parent, G19833 exhibits indeterminate plant growth and the inefficient parent, AND696, exhibits determinate plant growth. These differences were associated with differences in plant maturity in the population, such that the indeterminate plants averaged 5 days longer to reach maturity (Chapter 3, Table 7). Linkage group B1 has previously been shown to be important in common bean domestication, carrying both the *fin* gene and the *Ppd* gene for photoperiod sensitivity. QTL for domestication syndrome, including earliness and seed size traits have been identified on this linkage group, near these genes (Koinange et al., 1996). The importance of B1 near the *fin* gene suggests either a pleiotrophic effect of this gene on yield, or linkage of this gene with other genes important for yield. The observation that this region of the bean genome had an opposite effect on seed yield under high and low soil P levels may be an indication that growth habit itself is a key mechanism to improve tolerance to low P soils, perhaps by increasing days to maturity and allowing more time for P uptake from the soil. The cluster of domestication genes on B1 have pleiotrophic effects on other traits in common bean, including leafhopper resistance (Murray et al., 2004).

P use efficiency

P use efficiency (PUE) was defined in this study as the amount of seed yield per unit of P taken up by the plant. In 2005, PUE was found to be higher in the low P environment than in the high P environment (Figure 5; Chapter 3, Table 2). AND696 was at the low end of the distribution for this trait under low P, with an efficiency of 0.27 compared to the mean of the RILs at 0.51 (Figure 5). A

QTL for PUE was detected under high P on B11 that was positively contributed by G19833 (Table 1). PUE was not an important mechanism for tolerance to low soil P in the G19833/DOR364 population (Beebe et al., 2006). PUE may be more important for tolerance to low P soils in intra gene pool crosses like G19833/AND696 compared to inter gene pool crosses. Preliminary QTL studies with the P efficient bean line G21212 identified PUE as a factor in tolerance to low P soils (Miklas et al., 2006).

Seed P content

P content of the seed may serve as an indicator of tolerance to low P soils. In P limiting environments, greater levels of P in the seed may aid early plant growth (Lynch and Beebe, 1995). In the low P soil treatment, seeds contained less P than in the high P environments in both 2000 and 2005 (Figure 4, top; Chapter 3, Table 2). P content was correlated with seed yield ($r=0.30$ p value <0.0001) in 2005 in the low P environment, but was not correlated with yield in any other environment/year combination. QTL identified for this trait were identified on B1, B2 and B6 across environments and years, and in all cases an increase in seed P content was derived from G19833 (Table 1, Figure 6). Although the QTL for seed P content were consistent across years and environments, based on phenotypic correlation with seed yield it does not appear to be a valuable trait for selection to improve tolerance to low P soils.

Conclusions:

QTL were identified for root growth traits including root length density and root surface area. These QTL were derived from the P efficient parent,

G19833, and co-localized with QTL for root traits identified in previous studies. The QTL for root traits identified here did not, however, co-locate with QTL for P uptake, and no QTL for P uptake detected in the current study were derived from G19833.

QTL for yield in low P 2000 and high P 2005 mapped to the same region of the genome (B1), but were derived from different parents. Plant growth habit appeared to play an important role in determining yield in the low P environment in this population. QTL for root length density, root surface area, and seed yield were also found in the same area of B1. The clustering of QTL in this region suggests pleitropic effects of the *fin* gene on a diversity of plant traits, and raises questions to the value of selecting QTL in this region of the genome, to improve tolerance to low P soils. This assertion is supported by regression of yield data by soil P treatment, that identify determinate lines that yield better than indeterminate lines under both soil P levels (Chapter 3, Figures 3 and 4).

Genetic differences in P use efficiency were identified in this population. QTL across years and environments were identified for seed P content.

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Table 1. Putative QTL for seed traits identified from 75 recombinant inbred lines developed from AND696/G19833 cross grown under high (HP) and low (LP) soil phosphorus conditions in Darien, Colombia in 2000 and 2005.

Traits	Linkage group	Nearest marker†	LOD score‡	R^2 CIM¥	Additivity₣
Root length density					
LP 2005	B3	AG1	3.1*	0.14	-0.17
HP-2005	B1	AGTA01	4.7	0.18	-0.15
	D	AGTA02	4.5	0.20	-0.15
Root surface area					
HP 2005	B1	fin	5.6	0.21	-12.1
	D	AGTA02	4.2	0.24	-12.0
Shoot P					
LP 2005	B8	CTTA04	3.5	0.17	0.08
P uptake					
LP 2005	B8	O12.2200A	2.9*	0.12	1.54
HP 2005	B11	ACAC04	3.4	0.15	4.8
P use efficiency					
HP 2005	B11	R4.1400A	3.1*	0.12	-0.03
Seed yield					
HP 2005	B1	AGTA01	6.4	0.27	155
LP 2000	B1	ATA4	3.5*	0.17	-83
HP 2000	B6	BM170	4.3	0.45	-301
Seed P content					
LP 2005	B1	fin	8.0	0.28	-9.6
	B2	BM164	4.9	0.19	-7.2
	B6	GCTC03	4.5	0.13	-6.0
HP 2005	B1	fin	9.0	0.27	-13.9
	B6	GCTC03	6.8	0.19	-10.9
LP 2000	B2	BM164	3.7	0.13	-5.0
HP 2000	B1	ATA4	6.0	0.18	-12.6
	B2	BM164	5.5	0.15	-10.8
	B6	GCTC03	3.4	0.09	-8.4

† Primer information for each marker can be found in Tables A4-1, A4-2, and A4-3 of the Appendix.

‡ LOD: Log of odds

¥ Proportion of the phenotypic variance explained by QTL at test site using CIM (composite interval mapping).

₣ Effects of substituting a single allele from one parent to another. Positive values indicate that allelic contribution is from AND696 and negative from G19833.

* Indicates that the LOD score fell below the cutoff range of 1000 permutations at $p = 0.05$.

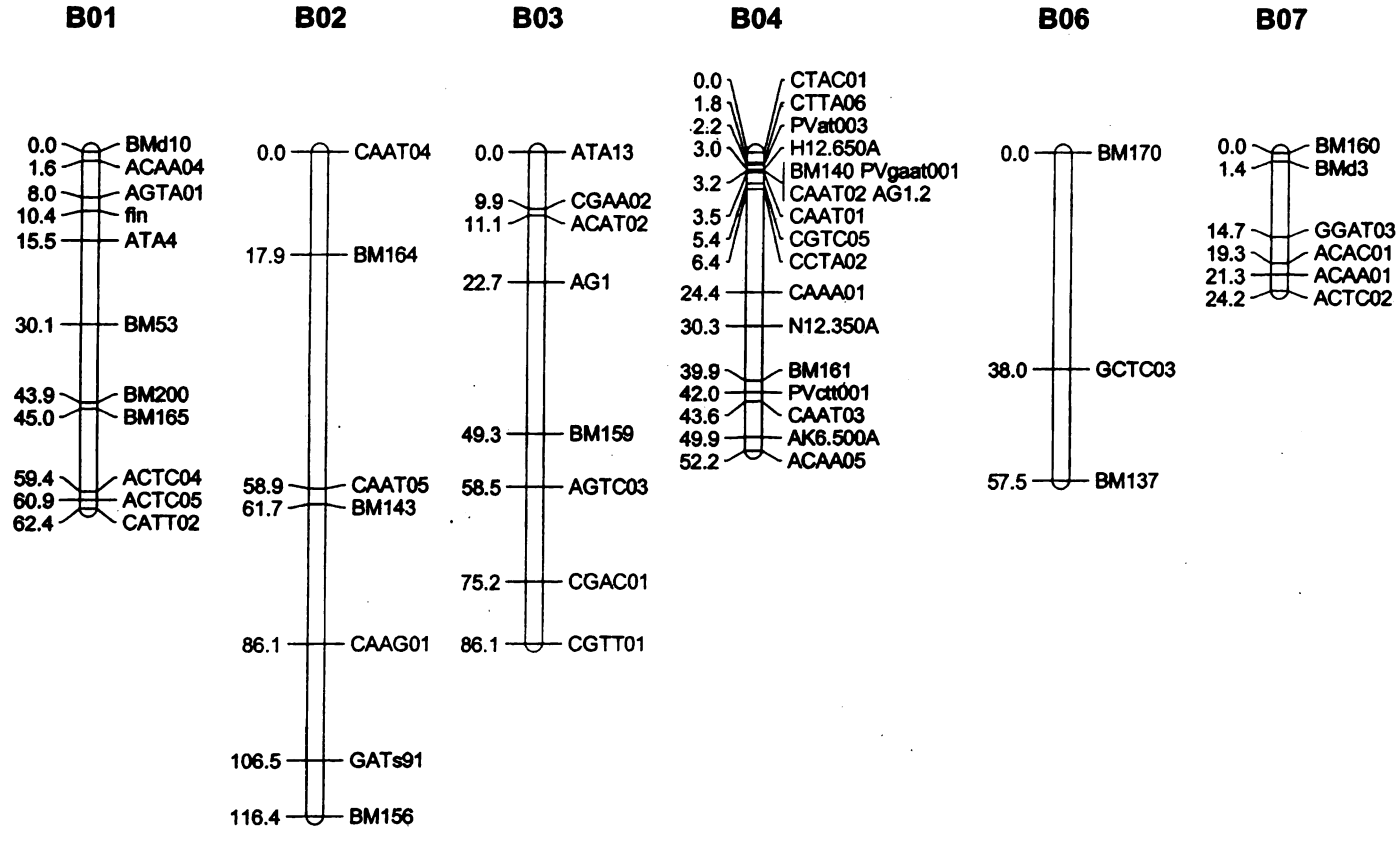
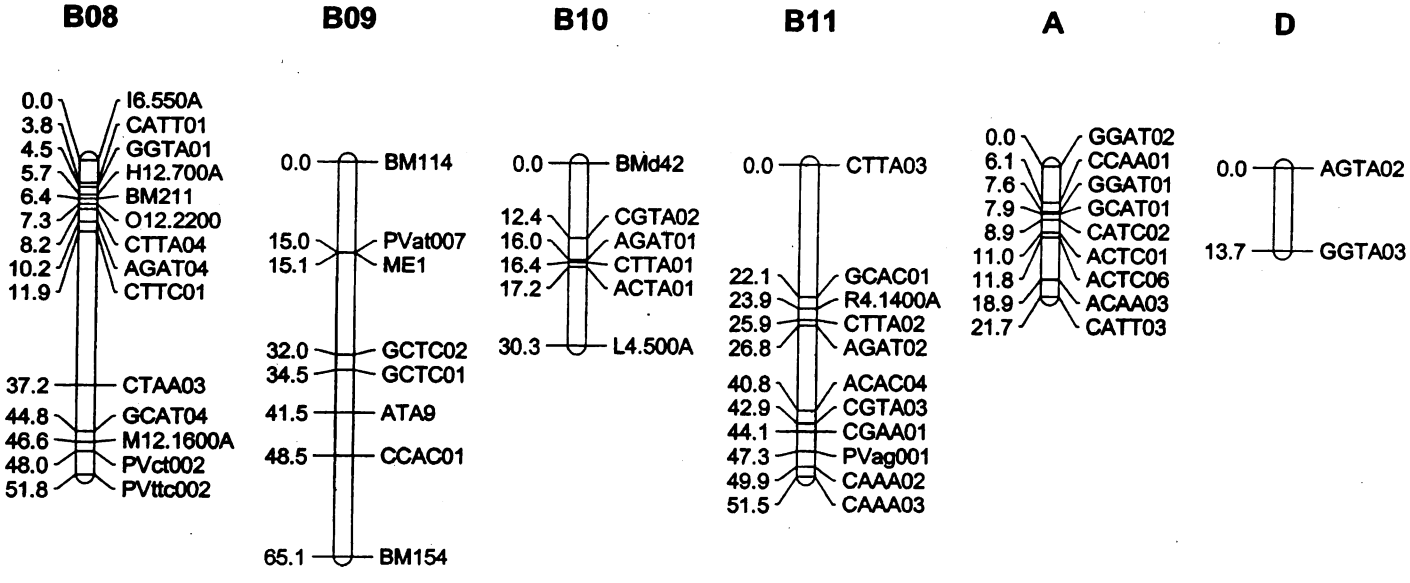


Figure 1. Common bean linkage map of AND696 by G19833 developed from 75 recombinant inbred lines of the $F_{5:7}$ population. The map contains RAPD, AFLP, SSR molecular markers and one phenotypic marker for a total of 633cM on 12 linkage groups. Primer information for markers at each locus can be found in Tables A4-1, A4-2, and A4-3 of the Appendix.

Figure 1 (cont'd).



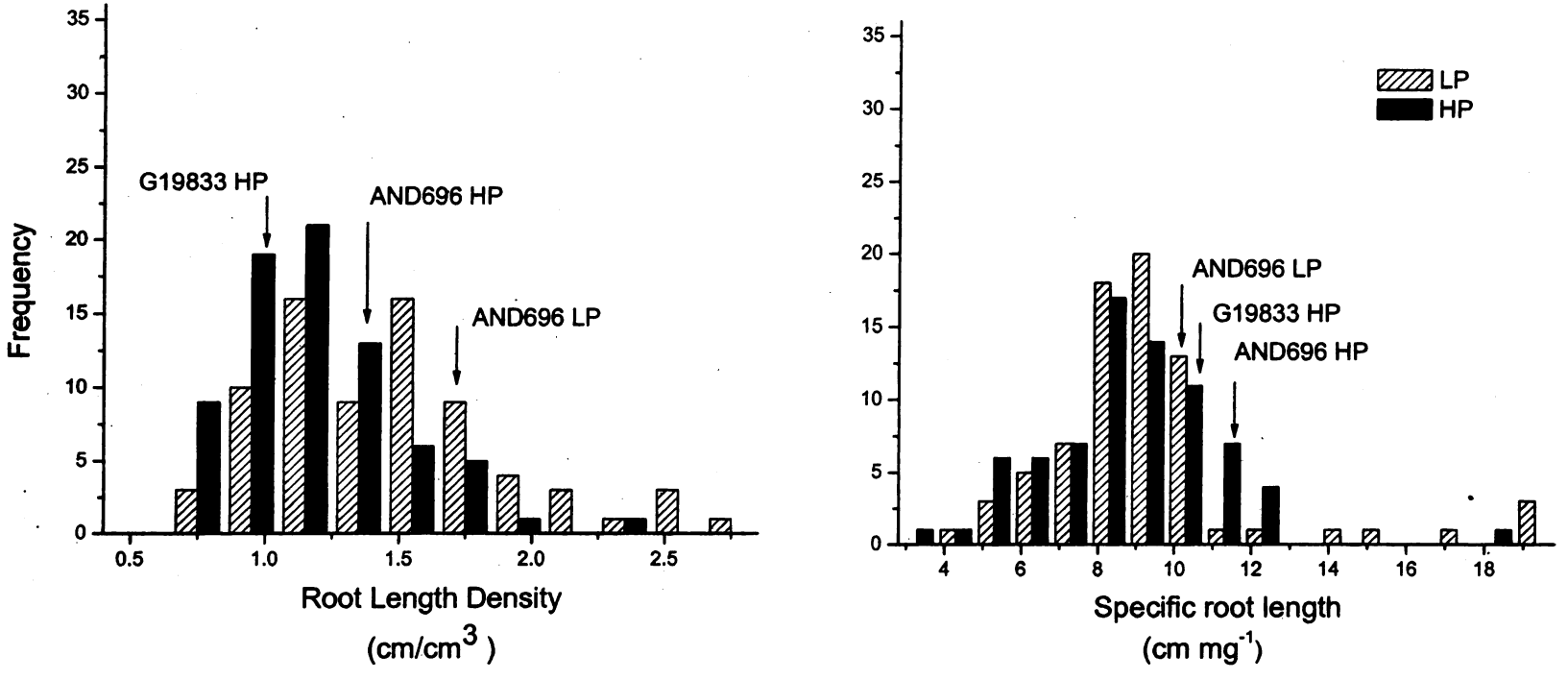
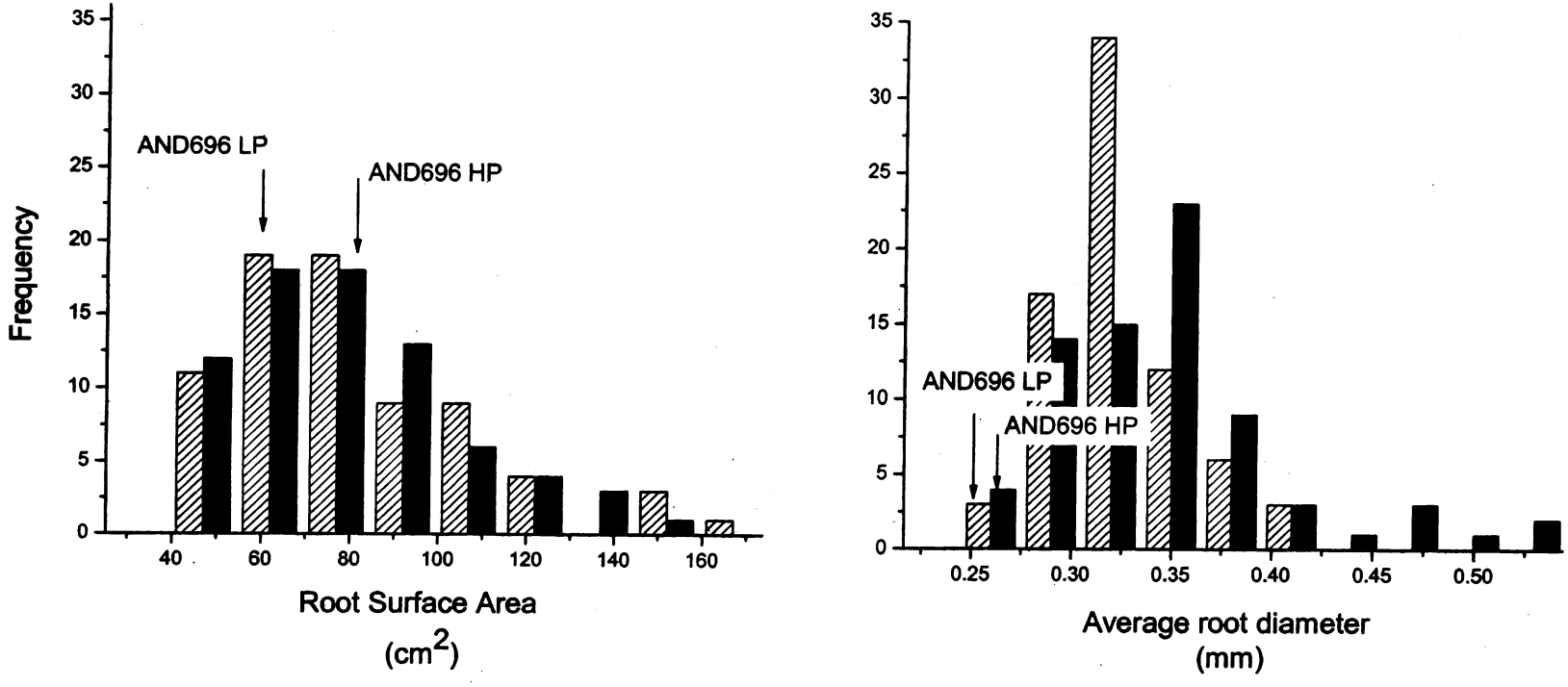


Figure 2. Frequency distribution of root traits in 75 recombinant inbred lines developed from a cross between AND696 and G19833 planted in Darien, Colombia in 2005 in high (HP) and low (LP) phosphorus soil. Arrows represent mean values of parental genotypes under different treatments (G19833 was not grown in 2005). Means are the average of 3 replications.

Figure 2 (cont'd).



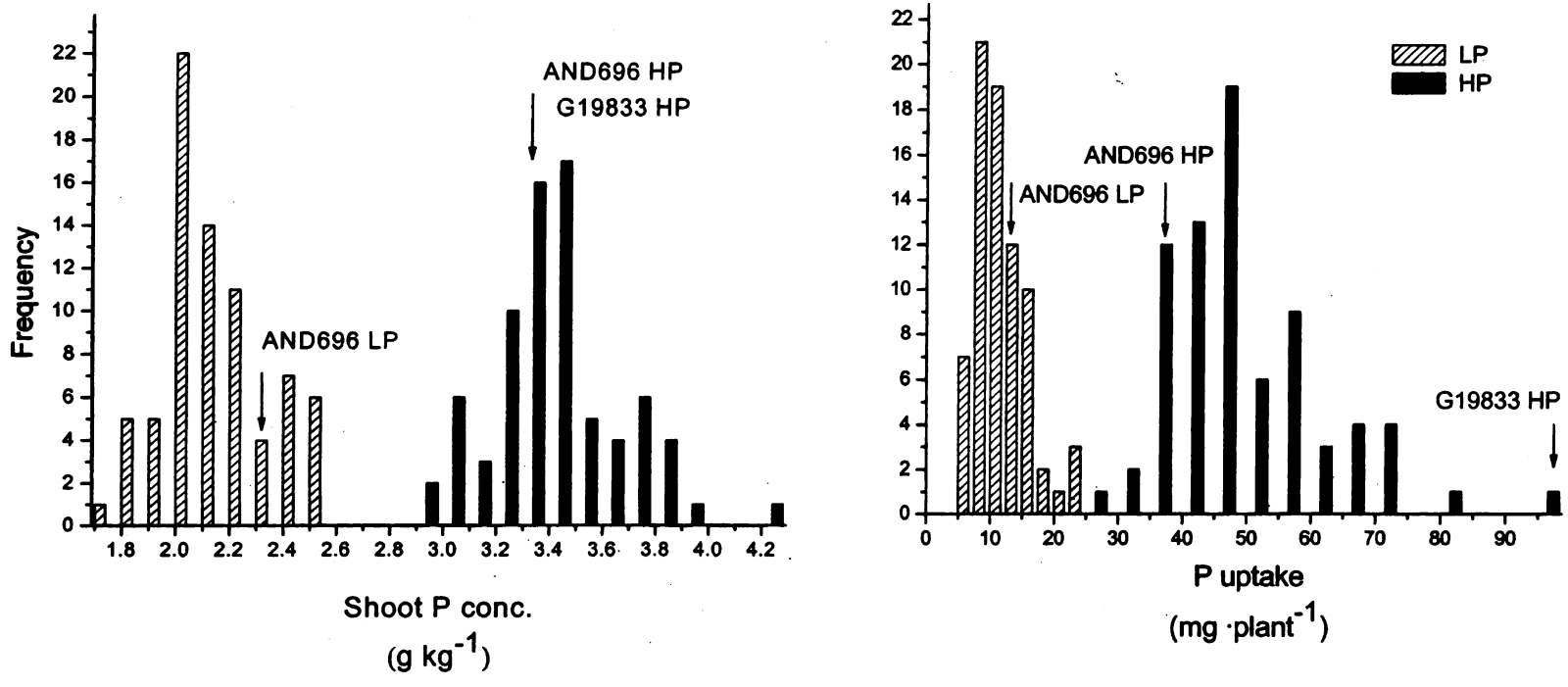


Figure 3: Frequency distribution of shoot phosphorus concentration and phosphorus uptake in 75 recombinant inbred lines developed from a cross between AND696 and G19833 planted in Darien, Colombia in 2005 in high (HP) and low (LP) phosphorus soil. Arrows represent mean values of parental genotypes under different treatments (G19833 was not grown in 2005). Means are the average of 3 replications.

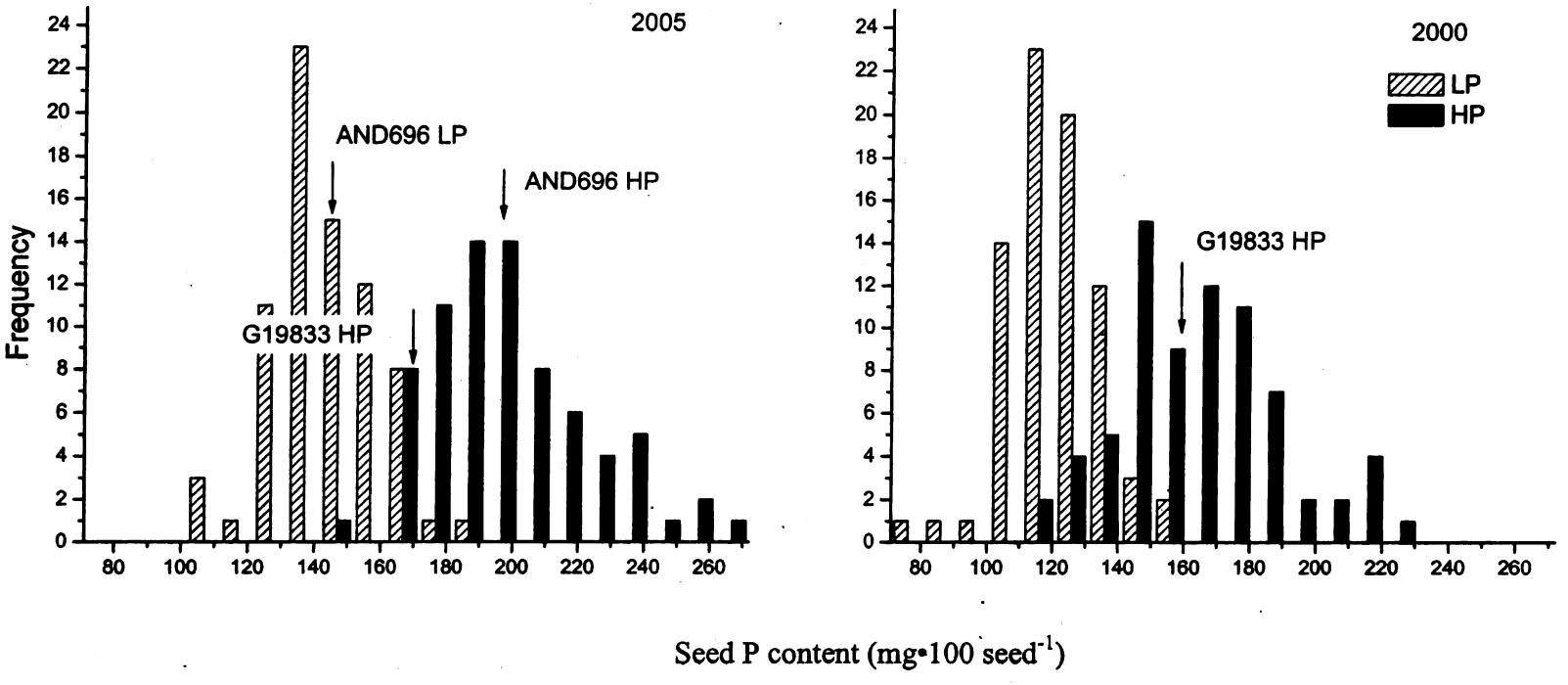
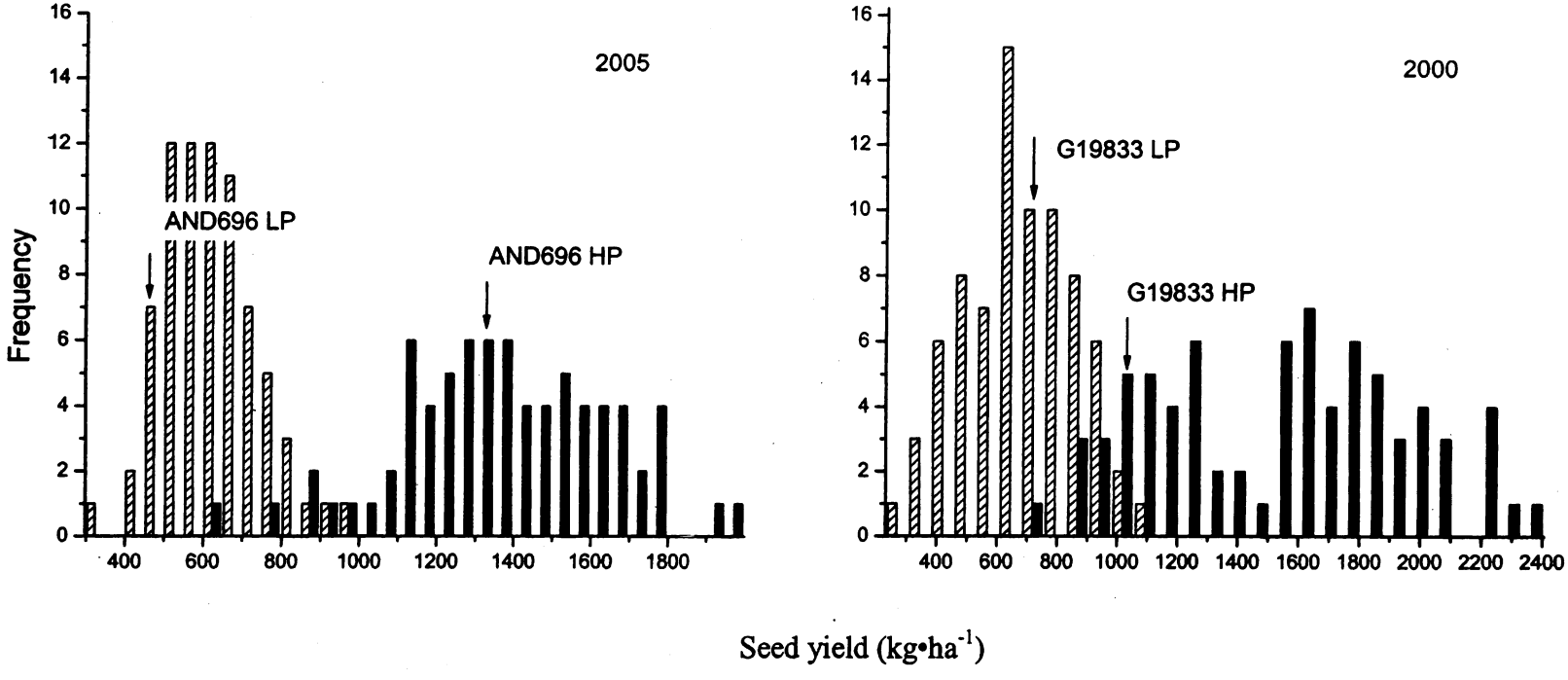


Figure 4. Frequency distribution of seed phosphorus content and seed yield in 75 recombinant inbred lines developed from a cross between AND696 and G19833 planted in Darien, Colombia in 2000 and 2005 in high (HP) and low (LP) phosphorus soil. Arrows represent mean values of parental genotypes under different treatments (G19833 was not grown in 2005 and AND696 was not grown in 2000). Means are the average of 3 replications.

Figure 4 (cont'd).



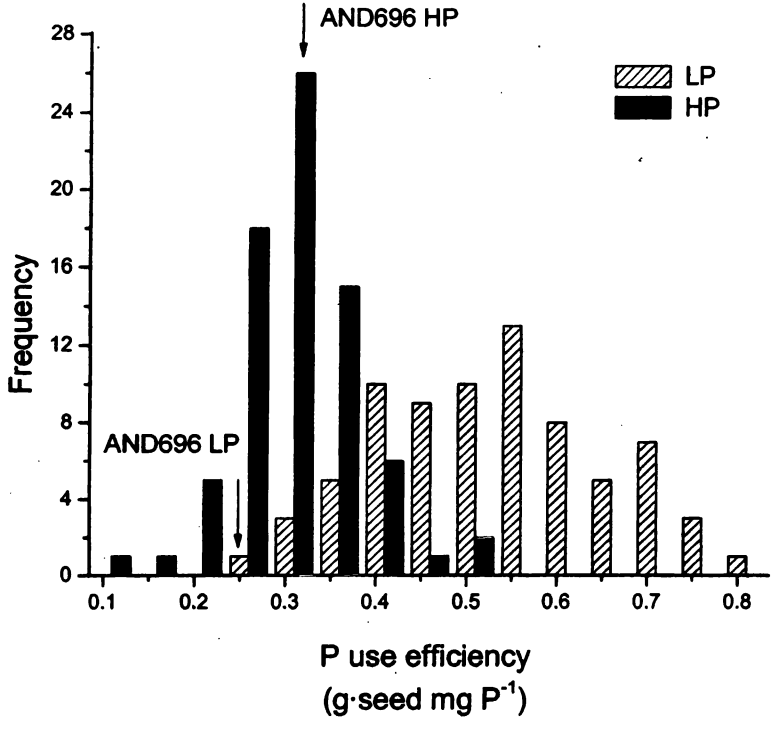


Figure 5. Frequency distribution of phosphorus use efficiency (defined in this study as the amount of seed yield per unit of P taken up by the plant) in 75 recombinant inbred lines developed from a cross between AND696 and G19833 planted in Darien, Colombia in 2005 in high (HP) and low (LP) phosphorus soil. Arrows represent mean values of parental genotypes under different treatments (G19833 was not grown in 2005). Means are the average of 3 replications.

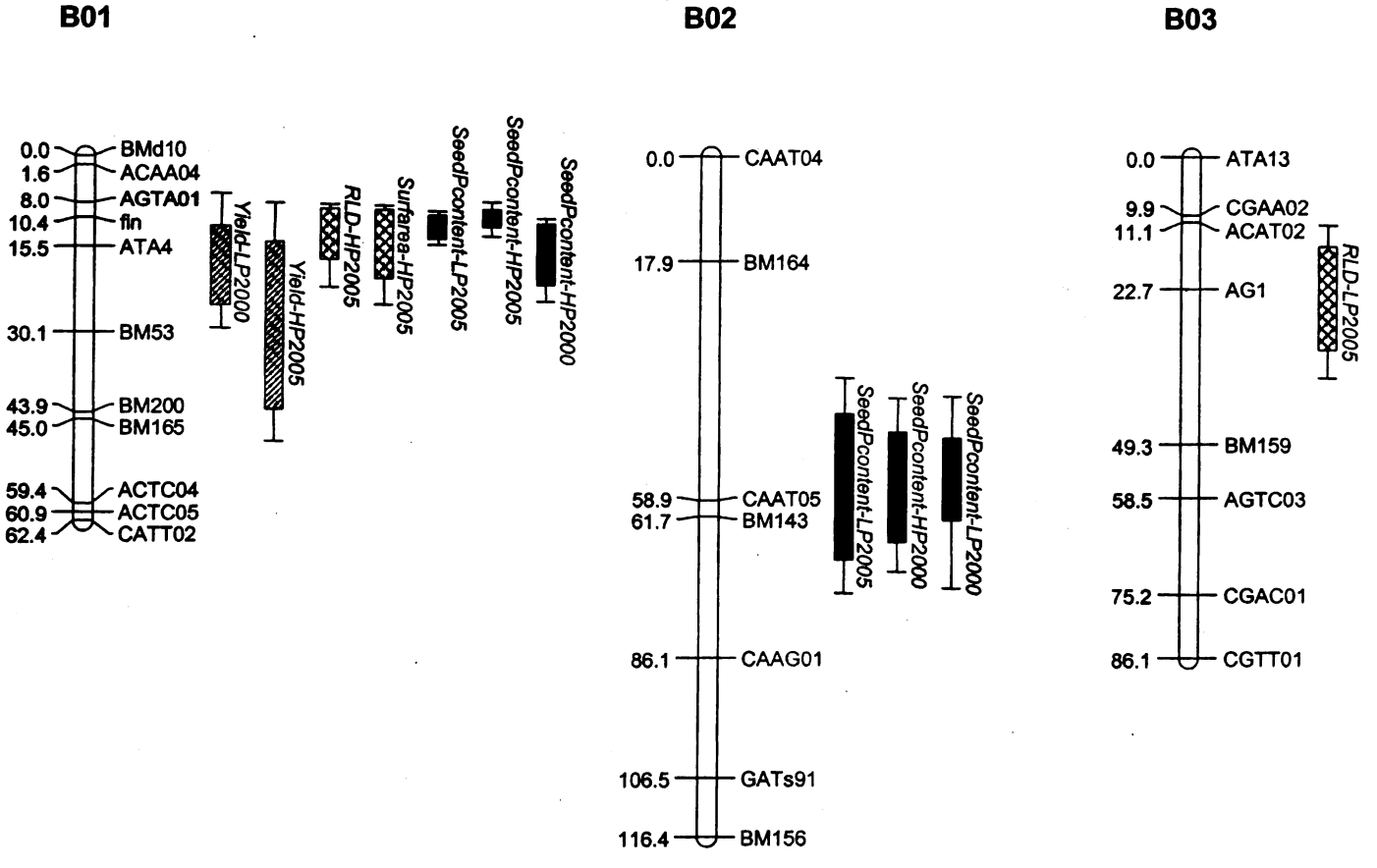


Figure 6. AND696/G19833 linkage map with QTL locations for seed yield, seed P content, root length density (RLD), root surface area (surfarea), shoot P concentration, P uptake, and P use efficiency (PUE). QTL are further identified by treatment, high P (HP) and low P (LP) and year, 2000 and 2005.

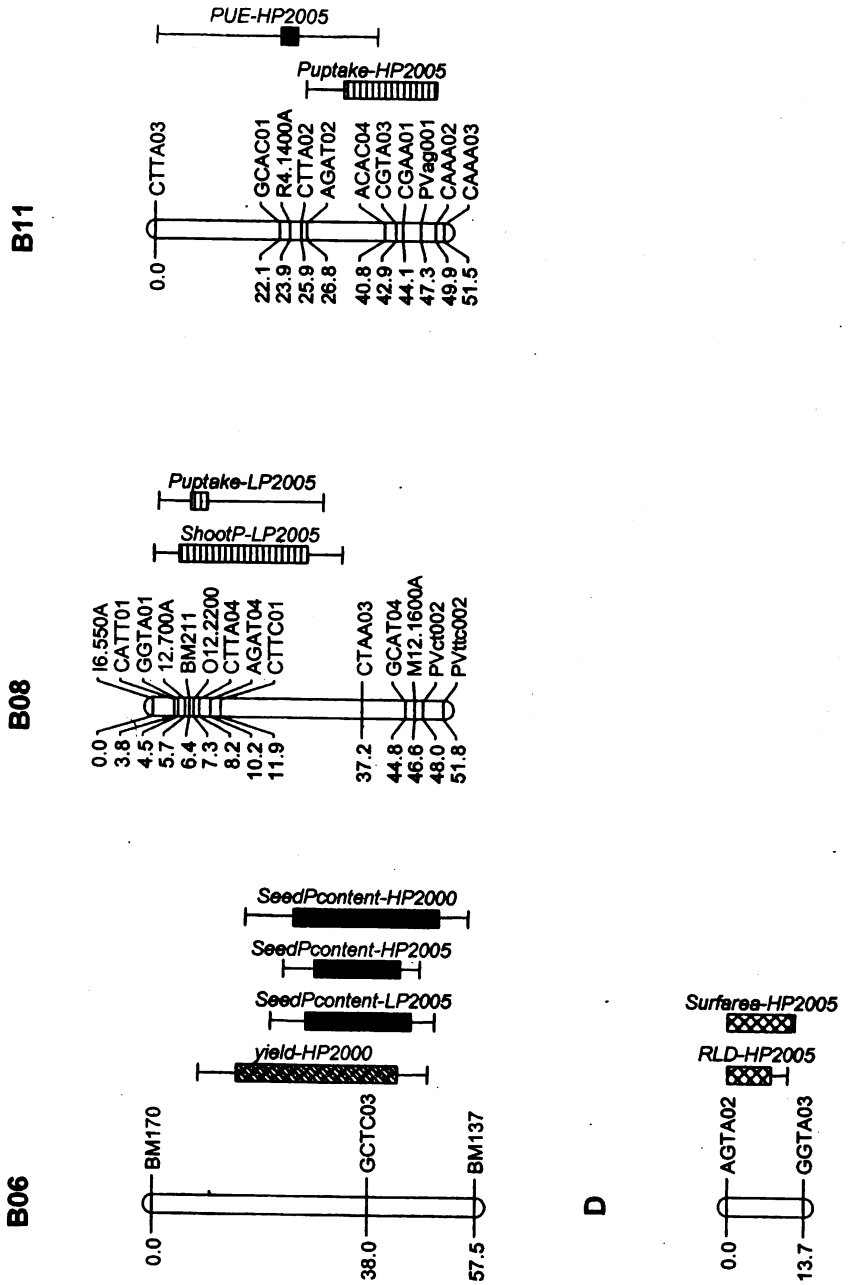


Figure 6 (cont'd).

Chapter 5: QTL analysis of Seed Nutrient Levels in an Andean Bean Population Segregating for Tolerance to Low Phosphorus Soils

Abstract:

Iron and zinc are essential micronutrients for human health that are often found in insufficient quantities in the diet, and biofortification of seed crops has been undertaken as a strategy to reduce micronutrient malnutrition. Bioavailability of Fe and Zn is reduced by seed phytate, the major form of phosphorus in the seed. Seed P levels also play a role in tolerance to low P soils, a major constraint of common bean production in the tropics. The objective of this study was to identify environmental and genetic variability for seed Fe, Zn and P concentrations in an $F_{5:7}$ recombinant inbred line (RIL) population developed from Andean common bean lines (AND696/G19833) with different tolerance to low P soils. Quantitative trait loci (QTL) analysis was conducted based on phenotypic data across two years and two environments (high and low soil P levels) and a previously described linkage map of AND696/G19833. Significant environmental and genetic variability for Fe, Zn, and P concentrations was identified population. Seed P levels were positively correlated with seed Fe and Zn levels. QTL for seed P levels explaining 17 to 55% of the total phenotypic variation depending on year and environment were identified across 4 linkage groups. QTL for Fe levels were identified across 5 linkage groups and QTL for Zn levels were identified on 2 linkage groups QTL for both Fe and Zn levels clustered on linkage groups B1 and B6 and also co-localized with QTL for seed P concentration on linkage group B1.

Introduction:

Micronutrient malnutrition is a major global health concern (Kennedy et al., 2003). More than half the population of the developing world receives inadequate micronutrient levels in their diets (Bouis, 2003). Two of the most widespread micronutrient deficiencies are that of Fe and Zn. Iron deficiency affects 3.7 billion people (Welch, 2002) and an estimated 49% of the human population is at risk for inadequate zinc in their diet (International Zinc Association, 2000).

Through the international, interdisciplinary research program HarvestPlus, a proposal was developed to alleviate micronutrient malnutrition by increasing the content of Fe and Zn in staple crops (Harvest Plus, 2004). Common bean (*Phaseolus vulgaris* L.), an important legume staple in much of the developing world, is one of the crops targeted for biofortification. Bean has long been recognized as a nutritionally valuable food for human nutrition. Although a cup of bean supplies 25% of the recommended daily allowance of iron and 15% RDA of zinc, the potential exists to develop varieties with two to three times the Fe and Zn content. Genetic variability for seed Fe concentration is 34 to >100 μg/g and 21 to 54μg/g for seed Zn concentration (Beebe et al., 2000).

Increased mineral content in the diet does not guarantee increased micronutrient status for the consumer. There are numerous compounds in plant-based diets that reduce micronutrient absorption by the body. Among these, phytate is the major determinate of Fe and Zn bioavailability in the diet (Lopez et al., 2002). Phytate (*myo*-inositol 1,2,3,4,5,6 hexa*kis*phosphate) is the major form

of phosphorus in cereal and legume seeds (Raboy, 1990). Phytic acid has 6 phosphate groups that can covalently bind to cations, especially K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, and Zn^{2+} (Tsao, 1997). Under human physiological conditions, some phytate complexes are insoluble, thus decreasing the bioavailability of phosphorus and cations, especially iron and zinc, stored as a part of the complex (Schlemmer et al., 1995). Numerous studies, using a variety of experimental techniques, have shown a correlation between high phytate diets and limited Fe and Zn absorbance in the gastrointestinal tract of humans and animals (Saha et al., 1994; House et al, 1982; Turnlund et al., 1984; Hunt et al., 1998; Zhou et al., 1992).

On one hand, P stored in legume seeds (in the form of phytic acid) can reduce micronutrient absorption by humans, but on the other hand, P is an element essential to plant growth that is often limited in the environment. In fact, P deficiency is one of the most widespread abiotic stresses affecting common bean production (Wortmann et al., 1998). Soil P deficiency often occurs in the same regions of the world plagued by micronutrient deficiencies in humans. Screening of common bean germplasm for ability to grown in low soil P conditions has resulted in the discovery of bean lines tolerant to low P soils (Lynch and Beebe, 1995; Yan et al., 1995 a,b; Beebe et al., 1997). QTL studies conducted with P efficient lines resulted in the identification of regions of the bean genome that play a role in tolerance to low P soils, and plant phenotypes associated with those regions (Beebe et al., 2006; Ochoa et al., 2006; Yan et al., 2004; Liao et al., 2004).

Since biofortification of common bean for Fe and Zn is underway and phosphorus plays such an important role in plant and human nutrition, an increased understanding of the genetics of tolerance to low P soils together with the genetics of seed P, Fe, and Zn concentration will be beneficial in the development of stress resistant and micronutrient fortified crops.

The objectives of this research were first, to measure seed P, Fe, Zn, and phytic acid levels in a population of recombinant inbred lines developed from common bean line G19833 tolerance to P soil and AND696, a line susceptible to low P soil. The second objective was to examine the relationship among these nutrients in the seed and tolerance to low P soils. The third objective was to identify QTL for seed nutrient levels in the RIL population.

Materials and Methods:

Seventy seven $F_{5:7}$ recombinant inbred lines were developed from a cross between two common bean lines from the Andean gene pool. The initial cross G19833 x AND696 was advanced to the $F_{5:7}$ generation by single seed descent and seed of the RILs was increased for field studies. The parent G19833 is a Peruvian landrace with an indeterminate (Type III) growth habit. G19833 has large yellow and red mottled seed with an average 100 seed weight of 41g. G19833 has also been identified as tolerant to low P soils (Yan et al., 1995a; Yan et al., 1995b). The parent AND696 is a CIAT improved line from the race Nueva Granada. It has a determinate growth habit (Type I) and has large red and white mottled seed with an average 100 seed weight of 51g. AND696 has been identified as susceptible to low phosphorus soils (CIAT, 2000).

In 2000 and 2005, the 77 RILs, G19833, AND696, and two check varieties, G4017 (Carioca) and G16140 were planted in Darien, Colombia in a 9x9 lattice design with three replications at two soil P levels, low P (45 kg/ha triple super phosphate) and high P (300 kg/ha triple superphosphate). Phenotypic data was collected on 75 of the 77 RILs. The soil of this site in an Andisol with a native soil P of 2 mg/kg based on bray II extraction method. Seed was hand-planted at 10 cm spacing in 4 row plots. Each row was 3 m in length. The middle two rows were planted to the genotypes of interest and the outer two rows were border rows of Mesoamerican cultivar BAT 477.

Seeds reached maturity at 76 to 98 days after planting. At maturity, seeds were hand-harvested. Seeds were dried and seed yield was determined at 18% moisture. Weight of 100 seed was measured at 18% moisture. A sub sample of seed was analyzed for P, Fe, and Zn concentration. Five grams of seed of each treatment and replication were cleaned with distilled water and dried. They were then freeze dried to remove all moisture. Freeze-dried samples were ground to a fine powder. Total seed P analysis was conducted according to Murphy and Riley (1962). Iron and Zn were extracted from seed with a Nitric-percloric (2:1) digestion according to the method of Benton and Jones (1989). Atomic absorption spectrometry was subsequently used to measure Fe and Zn concentration of the seed samples.

Phytic acid was measured in seed from the 2005 season by high performance liquid chromatography (HPLC) using a modified version of Graf and Dintis (1982). Sample preparation for PA analysis in bean seed was conducted

following the procedure of Lehrfeld (1989). PA was extracted from the samples by the addition of 20ml 0.65M HCl (trace element grade) to 0.500 g of sample. The acidified samples were mechanically agitated at 250rpm for 2 hours at 21°C. Samples were centrifuged at 4,000 rpm for 15 minutes. The supernant was collected and diluted 1:5 (v/v) with HPLC grade water. A 3 ml aliquot of the diluted sample (15 ml total) was passed through a Bond Elut strong anion exchange column (Varian, Walnut Creek, CA) for purification. The column was washed once with 5 ml 0.14 M HCl, followed by a 10 ml wash with 0.5M HCL. Phytic acid was eluted from the column with 3ml 2M HCl. The eluted fraction containing PA was freeze-dried and dissolved in 5mM sodium acetate. The dissolved sample was filtered through a 2μm filter. PA levels in each sample were quantified by HPLC with a refractive index detector. The column used for analysis was a Waters Symmetry C18 column (3.9mm x 150mm) (Waters, Milford, MA) heated to a temperature of 40°C. Sodium acetate (5mM) was used as the solvent at a flow rate of 1.4 ml min^{-1}. Phytic acid dodecasodium salt from corn (*Z. mays* L.) (Sigma, St. Louis, MO) was used as a standard to determine PA concentration. Data was converted to concentration on a dry weight basis.

Statistical analysis was conducted with SAS for Windows V8 (SAS Institute, Cary, NC). The command PROC GLM was used to determine treatment, genotype and interaction effects. Data was analyzed as a split plot with soil P level as the whole plot and plant genotype as the split plot. The command PROC CORR was used to determine Pearson correlation coefficients among variables. Effects of plant growth habit on measured variables were determined by means

comparisons of determinate vs. indeterminate growth habit types and differences were established using Tukey's test for significance. Bartlett test for variance homogeneity was conducted for growth habit because there were unequal number of determinate (56) and indeterminate (21) RILs.

A linkage map of the G19833/AND696 was developed using JoinMap JoinMap 3.0 for Windows (Van Ooijen and Voorrips, 2002) as described in the Materials and Methods section of Chapter 4 in this thesis. QTL analysis was conducted with the genetic map developed with the JoinMap program and with the phenotypic means for each RIL collected from the field study. The computer software program Windows QTL cartographer version 2.5 (Wang et al., 2006) was used to identify QTL for seed weight, seed P, Fe, Zn, and phytic acid concentrations. The Composite Interval Mapping (CIM) feature of Windows QTL cartographer employing the forward and backward regression model set to10cM window width was used to identify QTL. CIM analysis is based on maximum likelihood estimates and linear regression to identify QTL within marker intervals. Significant QTL were considered by defining the LOD score at p=0.01 after 1000 permutation tests.

Results:

Variability:

Seed weight: Seed weight was influenced by genotype and environment in the RIL population in both 2000 and 2005. There were no significant genotype x environment interactions (GxE) (Table 1). The mean 100 seed weight of the RILs was 2-3 g higher in the high P environment than the low P environment in

each year (Table 2). The ranges for seed weight overlapped between the environments, in each year (Figure 1). AND696 had larger seeds than G19833 in the high P environment (51.4 and 41.4 g per 100 seed, respectively) (Figure 1).

Seed P and phytic acid: Genotype and environment had a significant effect on seed P levels of the RILs in 2000 and 2005. In each year there was a significant GxE for this trait (Table 1). Concentration of seed P was higher in the high P than the low P environment each year (Table 2). The frequency distributions of seed P showed more variability for this trait in the high P environment than the low P environment (Figure 1). G19833 had higher seed P concentration in the high P environment than AND696 (Figure 1). Percent phytic acid, which was only measured in 2005, was significantly affected by soil P environment (Table 1). There was only a genotype effect for this trait observed in the low P environment (Table 2). In the high P soil environment, AND696 was at the low extreme of the RILs, at 1.46 %, whereas the range for the RILs ranged from 1.33 to 2.85 % (Figure 2).

Seed Fe and Zn: The concentrations of Fe and Zn in the seeds were significantly affected by genotype and environment. There was no significant G x E for either of these traits (Table 1). Iron levels were higher in the high P environment, whereas Zn levels were higher in the low P environment (Table 2). The inhibition of Zn uptake by high levels of soil P has been observed previously in common bean (Singh et al., 1988) and the phenomena is known as P induced Zn deficiency. There was greater variability for Fe levels in the population that

for Zn levels (Figure 3). The range of Fe levels was larger in 2005 than in 2000 (Figure 3).

Correlations: Correlations with seed weight and the other seed traits were not consistent across years. In 2000, seed weight was positively correlated with seed Fe and Zn in both high and low P treatments (Table 4). In 2005, seed weight was only positively correlated with seed Zn in the high P treatment (Table 3).

In each year and each P environment, seed P was positively correlated with seed Fe and Zn concentrations (Tables 3 and 4). Phytic acid % was also correlated with seed Fe and Zn in high and low P environments, but not as strongly as total seed P (Table 4). In 2005, seed Fe and Zn concentrations were weakly and significantly correlated with P uptake in the low P treatment, $r = 0.17$ and 0.12 respectively. In the high P treatment, only seed Zn was correlated with P uptake ($r = 0.32$).

Growth Habit: Seed weight, P, Fe, Zn, and phytic acid were each influenced by plant growth habit (Table 5). The indeterminate RILs had larger seed weights (49.6 g in high P and 46.2 g in low P) on average than the determinate RILs (46.8 g in high P and 43.7 g in low P) and also had greater concentrations of P, Fe, and Zn. There was an exception to this trend observed in 2000, under low P conditions where the determinate lines had higher seed P concentrations (Table 5). The differences in seed P concentration among years in may relate to differences in seed yield by growth habit in 2000 and 2005. In 2000 the average yield of the indeterminate lines was 707 $kg \cdot ha^{-1}$ under low P conditions and that of the determinate lines was 614 $kg \cdot ha^{-1}$. In 2005, there were

no differences in yield in the low P treatment based on growth habit. (data shown in Chapter 3). Since there were unequal number of determinate and indeterminate RILs in the population, Bartlett's test for variance homogeneity was conducted to identify bias that may have existed in trait analysis by growth habit. Most traits exhibited variance homogeneity although there were exceptions, including seed weight (high P, 2005), seed phytic acid (low P, 2005) and seed Zn (low P, 2005) (Appendix, Tables A4-4).

QTL identification:

Using composite interval mapping, 32 QTL were identified for 5 seed traits in 15 marker intervals on 8 linkage groups. Twenty-eight percent of the QTL were clustered on linkage groups B1 and affected more than one trait. Tweleve percent of the QTL were clustered on linkage groups B3. An additional 31% of the QTL were located on linkage group B6 and also affected multiple traits. Individual QTL explained 10 to 35% of the phenotypic variation, and total phenotypic variation explained for any one trait was 10 to 61% (Table 1).

Seed weight: A QTL for seed size was identified on linkage group B6 in each year and environment where the increased effect was contributed by G19833 (Table 6, Figure 4). This QTL explained 21 to 27% of the phenotypic variability in 2005, depending on environment and only 13 to 15% of the variability in 2000. An additional QTL for seed size was found in 2005 on B3 where the increased effect was contributed by AND696 (Table 6, Figure 4). QTL for seed size have previously been identified on B3 and B6 in a population developed from bean lines PC50 and XAN159 segregating for white mold resistance (Park et al., 2001).

A QTL for seed size, in the same region of B3 as that identified by Park et al. (2001) has also been found in the BAT 93/Jalo EEP558 population (BIC, 2006)

Seed P and phytic acid: QTL for seed P were found on B1 near the gene for plant growth habit. QTL found for seed P concentration on B1 explained 17 to 28% of the phenotypic variation for this trait depending on year and soil P level (Table 6, Figure 4). In 2000 and 2005 in the high P environment the increased effect was contributed by G19833, whereas in low P 2000 the increased effect was contributed by AND696.

A QTL for phytic acid on B8 mapped to same region as a QTL for total seed P in high P, 2005, where the increased effect was contributed by AND696 (Table 6, Figure 4). The QTL for % phytic acid explained only 10% of the phenotypic variation and the QTL for total seed P explained 20% of the phenotypic variation.

Seed Fe and Zn: QTL for both seed Fe and Zn were identified in the identical regions of B1 and B6 (Table 6) across years and environments. QTL found on B1 were near the *fin* gene, and also overlap with QTL for seed P concentration. G19833 was the source of the QTL identified for Fe and Zn concentration in the seed on B1. Those QTL found for seed Fe and Zn concentration on linkage group B6 explained 15 to 26% of the phenotypic variation. They also co-localized with QTL for seed weight. AND696 was the source of the increased effect of each of these QTL, conversely, G19833 was the source of the QTL for seed size identified in this region of linkage group B6 (Table 6).

Discussion:

Increasing the Fe and Zn concentration of seed crops is a major goal that bridges agriculture and human nutrition. In common bean, significant natural variability exists for seed Fe and Zn, making such a goal achievable through plant breeding (Beebe et al., 2000). Within the AND696/G19833 RIL population, Fe levels ranged from 38 to 79 mg $\cdot kg^{-1}$ and Zn levels ranged from 16 to 33 mg $\cdot kg^{-1}$. Understanding the genotype x environment interactions of these traits is essential to developing nutritious seeds for different environmental conditions. In this study, G x E was not significant for Fe or Zn seed concentration, suggesting that breeding for increased levels of these elements is possible under diverse soil P environments.

Mutational studies in plants have shown that many genes effect micronutrient accumulation and many remain to be discovered. QTL analysis will allow gene discovery to move forward for genes underlying natural variation in seed Fe and Zn levels (Ghandilyan et al., 2006). Overlapping QTL for Fe and Zn concentration were identified on two linkage groups in this study. This demonstrates the possibility of breeding for these traits simultaneously.

QTL for increased seed Fe and Zn concentration were located on the same region as a QTL for seed P concentration. These QTL were all contributed by G19833, which was the parent exhibiting tolerance to low P soils. This region of the genome (linkage group B1) may contain genes that improve tolerance to low P soils, as well, according to yield QTL data (Chapter 4, Table 1). Further research is required to determine the consequences of simultaneously selecting

increased seed Fe, Zn, and P levels on the bioavailability of these micronutrients in humans upon consumption.

In this study, only a single QTL for seed phytic acid was detected (Table 6). This QTL was not in the same region as QTL for seed Fe or Zn concentrations. In Arabidopsis, QTL for seed phytic acid levels have been separate from those for seed Fe and Zn levels suggesting it is possible to increase Fe and Zn levels without increasing phytate levels as well (Bentsink et al., 2003; Ghandilyan et al., 2006).

Since many of the QTL detected in this study for each of the traits co-localized it is interesting to consider the physiological role of the genes underlying these QTL. Could these genes be involved in seed transport or in uptake of nutrients by the plant roots? Iron uptake by pea (*Pisum sativum*) has been shown to increase during seed filling as compared to earlier in plant development. During this time, a constant source of micronutrients in required in the xylem stream for xylem-to-phloem exchange to occur, and for micronutrients to arrive in the seed (Grusak et al., 1999).

QTL for seed Fe, Zn, and P on B1 co-localized with the *fin* gene (Figure 4). This is the single gene in bean responsible for determinacy (Norton, 1915). The QTL for increased levels of each of these elements was conferred by the indeterminate parent (G19833). That indeterminate plants generally continue root growth beyond flowering whereas determinate plants do not (Huyghe, 1998), which may explain why this region of the genome influenced these traits for increased nutrients in the seed.

Conclusions:

Significant variability for seed weight, P, Fe and Zn concentrations was present in the AND696/G19833 population. Seed P concentration was correlated with seed Fe and Zn concentrations. QTL for these traits were found to co localize on linkage group B1. Seed Fe and Zn concentrations were also correlated and QTL for these traits co-localized not only to linkage group B1, but to B6 as well. The absence of GxE interactions for seed Fe and Zn levels is promising in terms of future development of lines with increased levels of these nutrients when planted in diverse environments.

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Table 1: Analysis of variance of seed traits in a population of 75 recombinant inbred lines from the AND696/G19833 population field grown in Darien, Colombia in 2000 and 2005 in two environments: high and low soil phosphorus.

		Mean Squares				
Source	**df**	**Seed weight**	**Seed P**	**Seed Fe**	**Seed Zn**	**Seed phytic acid**
2005						
Genotype (G)	74	127^{***}	0.29^{***}	205^{***}	26^{***}	0.11^{ns}
Environment (E)	1	1094^{***}	95.13^{***}	3404^{***}	943^{***}	15.49^{***}
G x E	74	7.3^{ns}	0.10^{*}	36^{ns}	7^{ns}	0.10^{ns}
2000						
Genotype (G)	74	104^{***}	0.26^{***}	133^{***}	20^{***}	-----
Environment (E)	1	540^{***}	72.07^{***}	459^{***}	862^{***}	-----
G x E	74	13^{ns}	0.18^{***}	43^{ns}	6^{ns}	-----

^{ns} indicates not significant
*** Indicates significance at $P < 0.05$.**
****Indicates significance at $P < 0.01$.**
***** Indicates significance at P<0.001**

Table 2: Seed traits in high (HP) and low (LP) phosphorus soils conditions for parents AND696 and G19833 and the means and ranges of 75 recombinant inbred lines developed from the parents. The experiment was planted in 2000 and 2005 in Darien, Colombia. Mean values presented (n=3). P value indicates level of significant genotypic differences among the RILs for each trait.

		Parents		Recombinant Inbred Lines		
Traits	**P level**	**AND696**	**G19833**	**mean**	**range**	**P value**
				2005		
Seed weight	**HP**	51.4	41.4	47.6	39.5-59.9	<.0001
(g ·100 seed^{-1})	**LP**	44.7	-------	44.5	33.3-56.5	<.0001
Seed P	**HP**	3.84	4.00	4.05	3.56-4.85	<.0001
(g ·kg^{-1})	**LP**	3.28	-------	3.14	2.63-3.51	<.0001
Seed Fe	**HP**	62.5	61.6	58.3	39.02-78.75	<.0001
(mg ·kg^{-1})	**LP**	48.6	-------	52.8	37.92-67.66	<.0001
Seed Zn	**HP**	20.2	21.0	22.7	18.74-30.35	0.1550
(mg ·kg^{-1})	**LP**	26.3	-------	25.6	20.18-32.83	<.0001
Seed phytic	**HP**	1.46	-------	1.77	1.33-2.85	0.2214
acid (%)	**LP**	1.26	-------	1.38	1.09-2.46	0.0272
				2000		
Seed weight	**HP**	------	34	44.94	35.5-56.75	<.0001
(g ·100 seed^{-1})	**LP**	------	------	42.78	30.5-55	<.0001
Seed P	**HP**	------	4.77	3.54	2.84-4.98	<.0001
(g ·kg^{-1})	**LP**	------	------	2.70	2.36-3.04	<.0001
Seed Fe	**HP**	------	78.45	64.97	53.3-76.9	<.0001
(mg ·kg^{-1})	**LP**	------	------	63.01	53.1-75.6	<.0001
Seed Zn	**HP**	------	25.43	20.60	16.3-27.4	<.0001
(mg ·kg^{-1})	**LP**	------	------	23.59	18.9-29	<.0001

Table 3. Phenotypic correlations among seed traits in a population of 75 recombinant inbred lines from the AND696/G19833 population field grown in Darien in 2005 in high and low soil phosphorus. The values above the diagonal line are under low soil P and the values below the diagonal line are under high soil P.

LP ---------- HP	**Seed P**	**Seed phytic acid**	**Seed weight**	**Seed Fe**	**Seed Zn**
Seed P	----------	0.35***	-0.05	0.53***	0.50***
Seed phytic acid	0.27***	----------	0.07	0.16**	0.26***
Seed weight	0.04	-0.09	----------	0.10	0.10
Seed Fe	0.31***	0.16**	0.00	----------	0.53***
Seed Zn	0.48***	0.10	0.23***	0.31***	----------

*** Indicates significance at $P < 0.05$.**
****Indicates significance at $P < 0.01$.**
***** Indicates significance at P<0.001**

Table 4. Phenotypic correlations among seed traits in a population of 75 recombinant inbred lines developed fromAND696/G19833 and field grown in Darien in 2000 in high and low soil phosphorus. The values above the diagonal line are under low soil P and the values below the diagonal line are under high soil P.

LP ---------- HP	**Seed P**	**Seed weight**	**Seed Fe**	**Seed Zn**
Seed P	----------	-0.13*	0.18**	0.28***
Seed weight	0.26***	----------	0.14**	0.14**
Seed Fe	0.39***	0.15**	----------	0.17***
Seed Zn	0.65***	0.26***	0.45***	----------

*** Indicates significance at $P < 0.05$.**
****Indicates significance at $P < 0.01$.**
***** Indicates significance at P<0.001**

Table 5. Seed traits in a population of 75 recombinant inbred lines developed from an AND696/G19833 cross and grown under high and low soil phosphorus conditions in Darien, Colombia in 2000 and 2005, grouped and averaged by plant growth habit of which there were two categories, indeterminate (Ind.) and determinate (Det.). Mean values presented (n=3).

Traits	**High P**		**Low P**	
	Ind.	**Det.**	**Ind.**	**Det.**
		2005		
Seed weight (g ·100 seed^{-1})	49.6 ***a***†	46.8 ***b***	46.2 ***a***	43.7 ***b***
Seed P (g ·kg^{-1})	4.23 ***a***	3.97 ***b***	3.2 ***a***	3.1 ***b***
Seed Fe (mg ·kg^{-1})	63.3 ***a***	56.1 ***b***	55.7 ***a***	51.4 ***b***
Seed Zn (mg ·kg^{-1})	24.22 ***a***	21.96 ***b***	27.4 ***a***	24.7 ***b***
Seed phytic acid (%)	1.78 ***a***	1.76 ***a***	1.46 ***a***	1.34 ***b***
		2000		
Seed weight (g ·100 seed^{-1})	46.8 ***a***	44.2 ***b***	43.6 ***a***	42.4 ***a***
Seed P (g ·kg^{-1})	3.7 ***a***	3.5 ***b***	2.6 ***a***	2.8 ***b***
Seed Fe (mg ·kg^{-1})	67.4 ***a***	64.0 ***b***	63.4 ***a***	62.8 ***a***
Seed Zn (mg ·kg^{-1})	22.3 ***a***	19.9 ***b***	24.4 ***a***	23.2 ***b***

† Significant differences are based on Tukey tests and are at alpha = 0.05. Values that do not share the same letter are significantly different. Tests should be read across rows and within each phosphorus treatment level, High P or Low P.

Table 6. Putative QTL for seed traits identified from 75 recombinant inbred lines developed from an AND696/G19833 cross grown under high (HP) and low (LP) soil phosphorus conditions in Darien, Colombia in 2000 and 2005.

Seed traits	Linkage group	Nearest marker	LOD score[†]	R^2 CIM[‡]	Additivity[F]
Seed wt.					
LP 2005	B3	AGTC03	5.6	0.18	2.1
	B6	GCTC03	6.7	0.21	-2.4
HP 2005	B3	AGTC03	8.4	0.31	2.7
	B6	BM170	7.3	0.27	-2.6
LP 2000	B3	AGTC03	2.7*	0.16	1.9
	B6	GCTC03	3.8	0.15	-1.9
HP 2000	B2	BM164	3.9	0.35	-2.8
	B6	GCTC03	3.8	0.13	-1.8
Seed P					
LP 2005	B3	AGTC03	4.3	0.22	-0.09
HP 2005	B1	AGTA01	4.3	0.17	-0.14
	B4	CGTC05	3.6	0.18	0.13
	B8	I6.550A	4.3	0.20	0.14
LP 2000	B1	AGTA01	4.3	0.17	0.08
HP 2000	B1	fin	6.9	0.28	-0.22
	B2	BM143	3.1	0.10	-0.13
	A	ACTC06	3.6	0.14	-0.15
Phytic acid					
LP 2005	B8	I6.550A	2.3*	0.10	0.06
Seed Fe					
LP 2005	B1	BMd10	5.4	0.18	-2.4
	B6	BM170	6.9	0.26	2.7
HP 2005	B1	AGTA01	8.1	0.24	-3.9
	B6	BM170	5.3	0.15	2.8
	B9	ATA9	5.1	0.14	2.8
	A	GGAT02	3.3	0.08	-2.2
LP 2000	B8	M12.1600A	4.0	0.15	1.86
HP 2000	B1	BM165	2.6*	0.10	-2.1
	B6	BM170	4.5	0.22	3.2
Seed Zn					
LP 2005	B1	fin	7.5	0.18	-1.2
	B6	BM170	8.2	0.24	1.3
HP 2005	B1	fin	6.8	0.31	-1.3
LP 2000	B1	BMd10	2.8*	0.10	-0.7
	B6	BM170	4.1	0.16	0.9
HP 2000	B1	fin	7.8	0.35	-1.5

Table 6 (cont'd).

† LOD: Log of odds

‡ Proportion of the phenotypic variance explained by QTL at test site using CIM (composite interval mapping).

F Effects of substituting a single allele from one parent to another. Positive values indicate that allelic contribution is from AND696 and negative from G19833.

* Indicates that the LOD score fell below the cutoff range of 1000 permutations at $p = 0.05$.

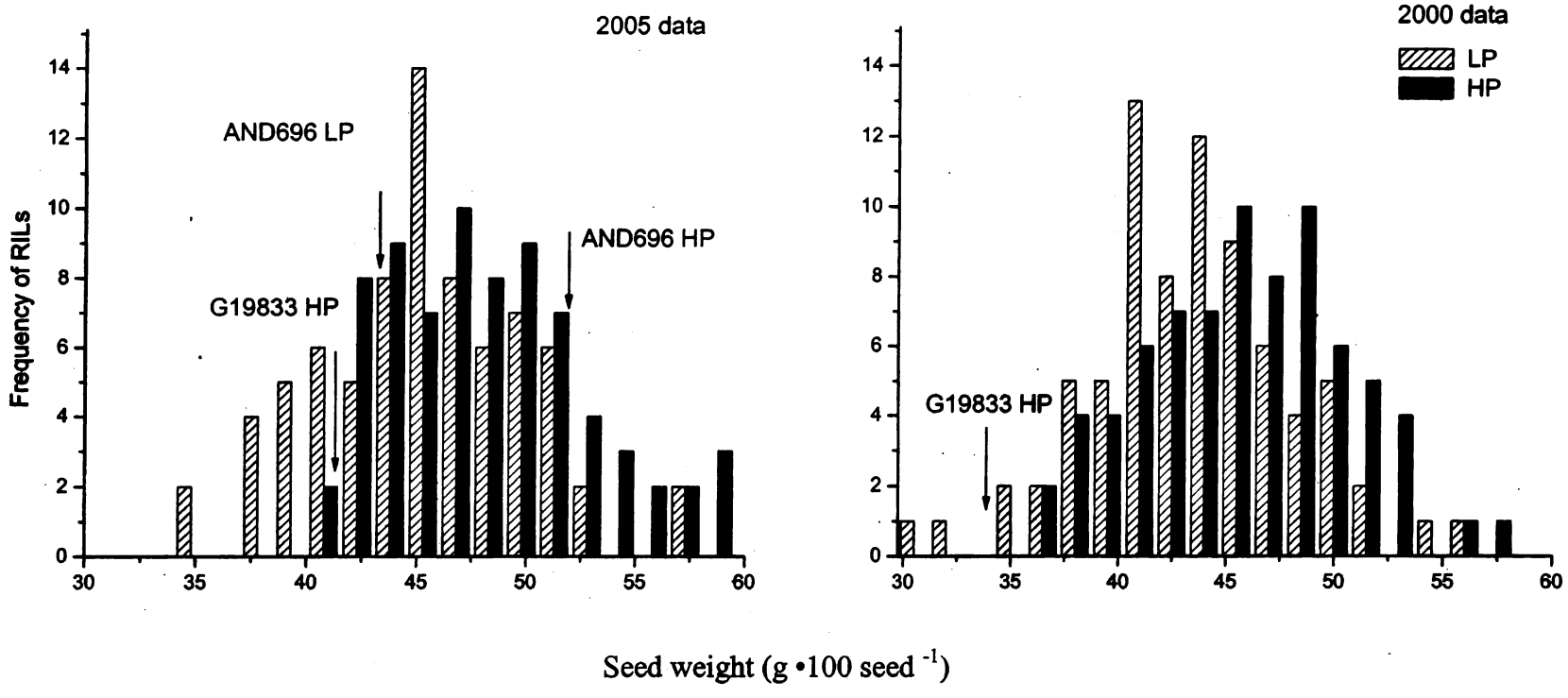
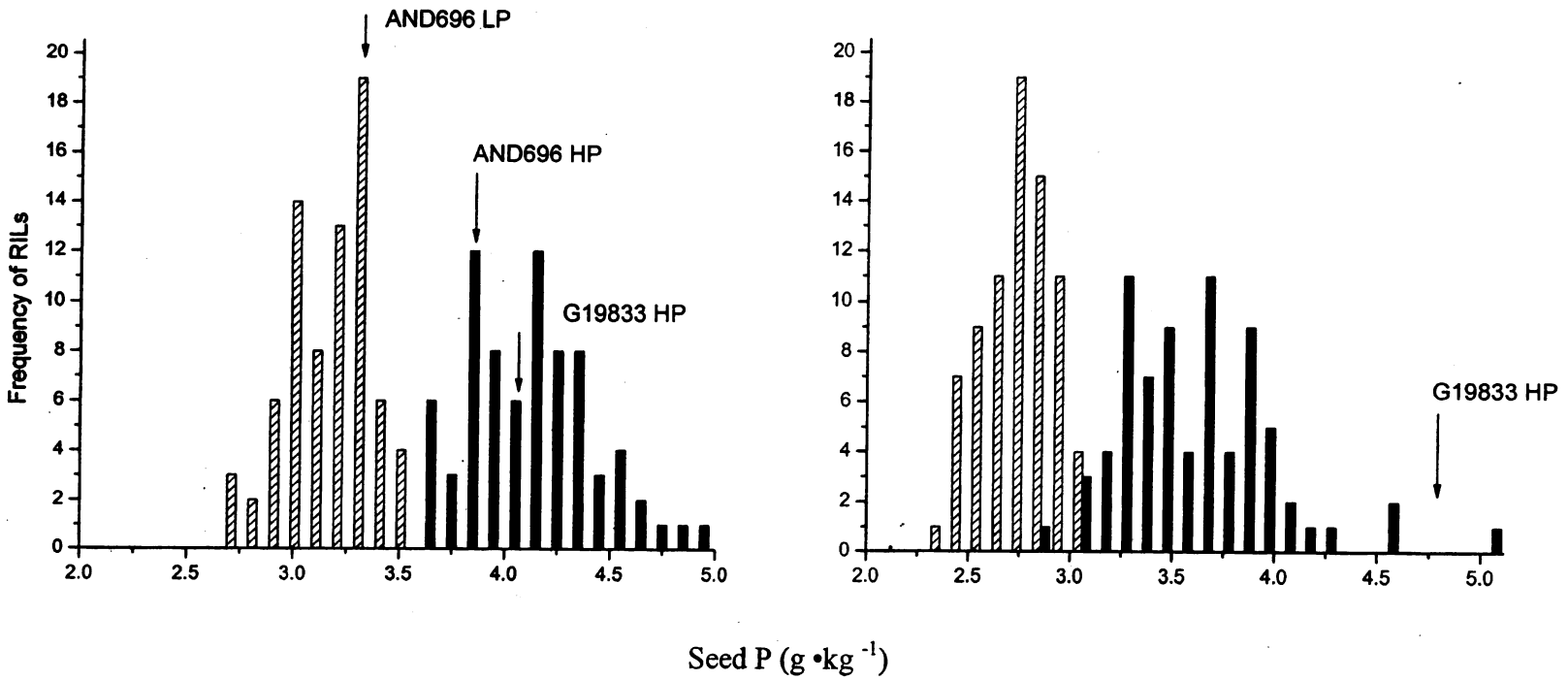


Fig. 1. Frequency distributions for seed weight and seed P in 75 recombinant inbred lines (RILs) from the population AND696/G19833 grown in Darien Colombia in 2000 and 2005 under high (HP) and low (LP) soil phosphorus levels. Arrows represent mean values of parental genotypes under different treatments (G19833 was not grown in 2005 and AND696 was not grown in 2000). Values are the average of 3 replications. Graphs on the left are for 2005 and those on the right are for 2000.

Fig. 1 (cont'd).


AND696 LP
AND696 HP
G19833 HP
G19833 HP
Frequency of RILs
Seed P (g •kg -1)

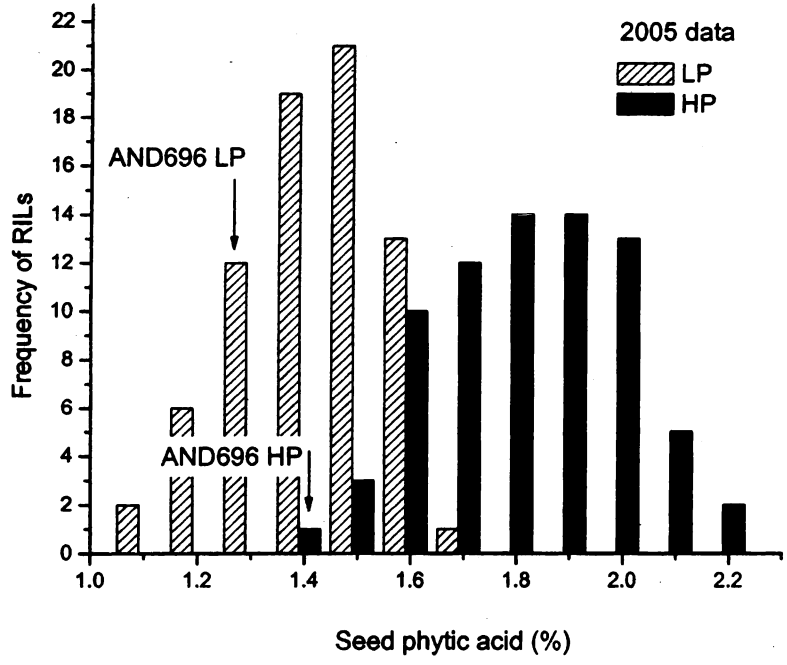


Fig. 2. Frequency distributions for seed phytic acid in 75 recombinant inbred lines (RILs) from the population AND696/G19833 grown in Darien Colombia in 2005 under high (HP) and low (LP) soil phosphorus levels. Arrows represent mean values of parental genotypes under different treatments (G19833 was not grown in 2005). Values are the average of 3 replications.

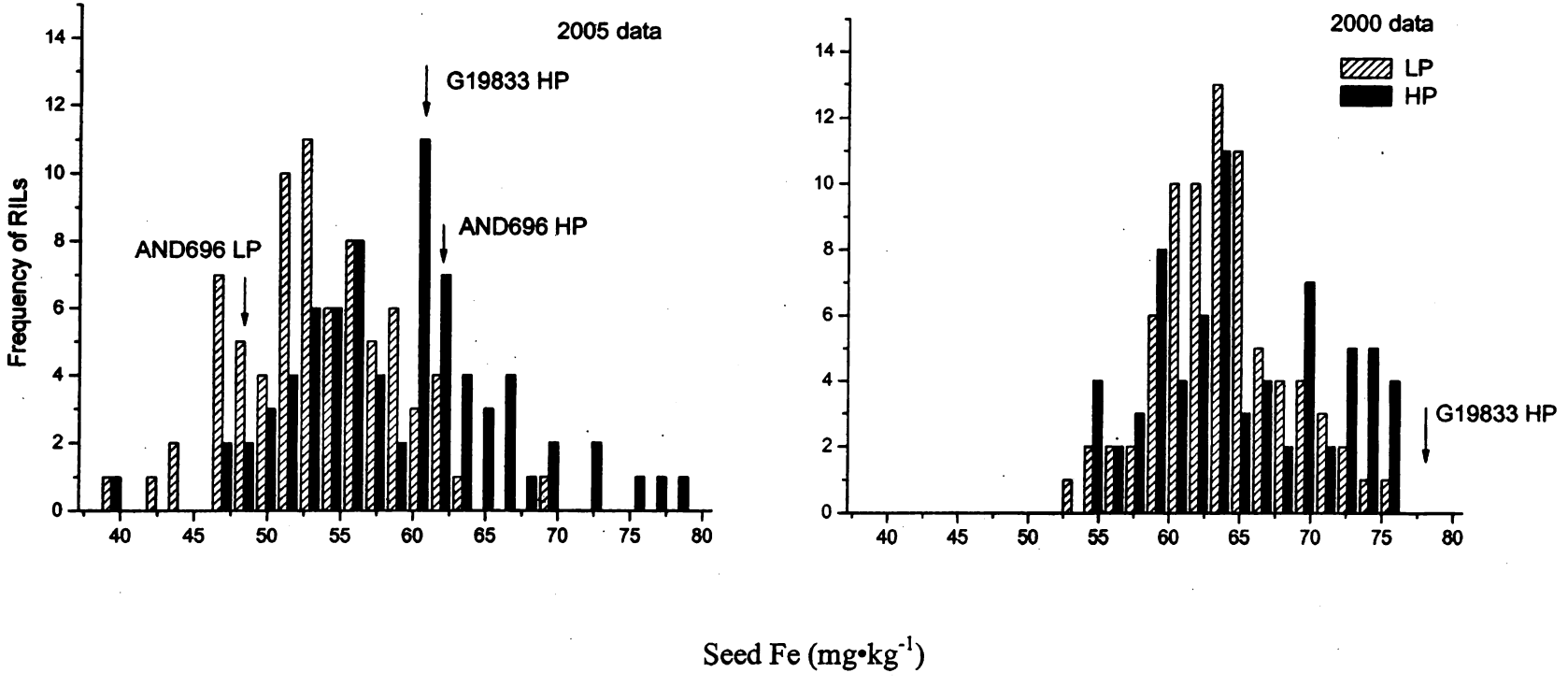
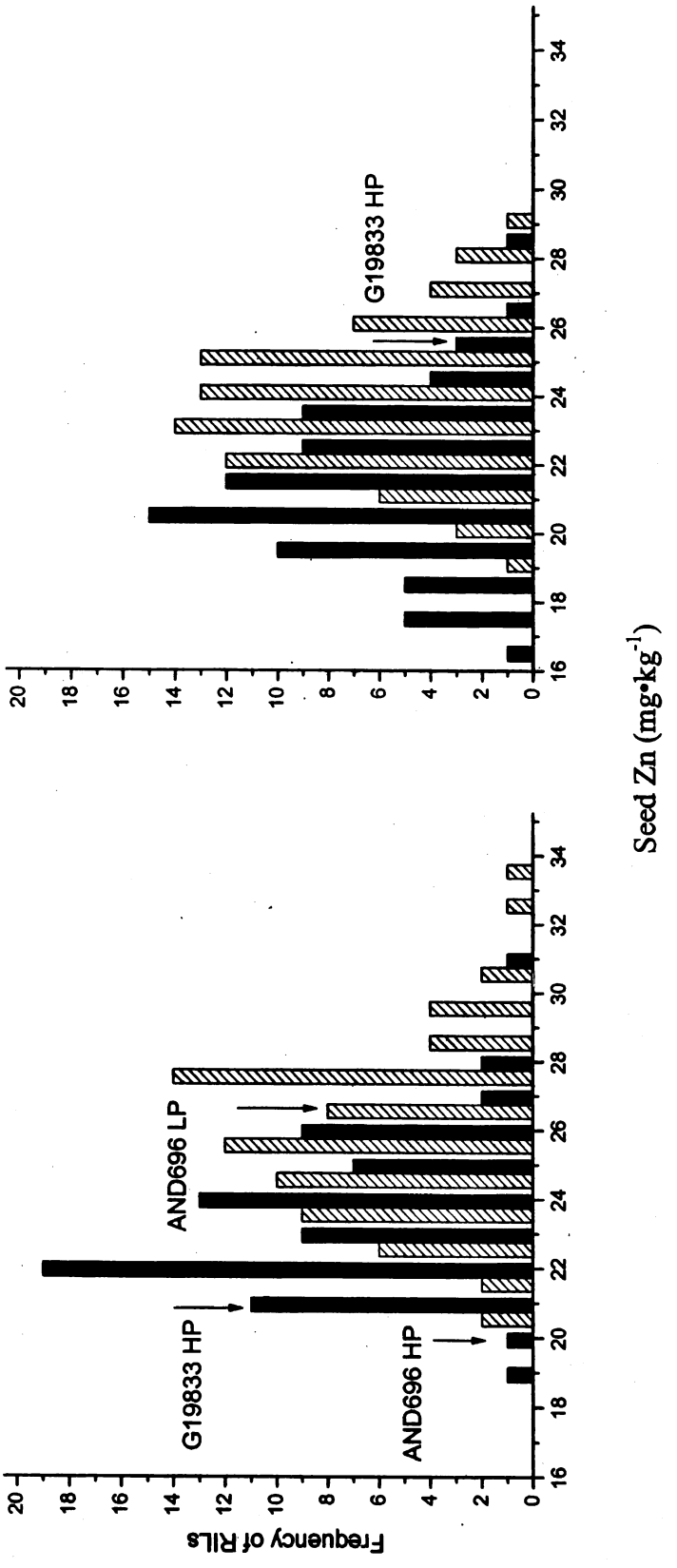


Fig. 3. Frequency distributions for seed Fe and Zn in 75 recombinant inbred lines (RILs) from the population AND696/G19833 grown in Darien Colombia in 2000 and 2005 under high (HP) and low (LP) soil phosphorus levels. Arrows represent mean values of parental genotypes under different treatments (G19833 was not grown in 2005 and AND696 was not grown in 2000). Values are the average of 3 replications. Graphs on the left are for 2005 and those on the right are for 2000.

Fig. 3 (cont'd).


Frequency of RILs
G19833 HP
AND696 HP
AND696 LP
G19833 HP
Seed Zn (mg*kg-1)

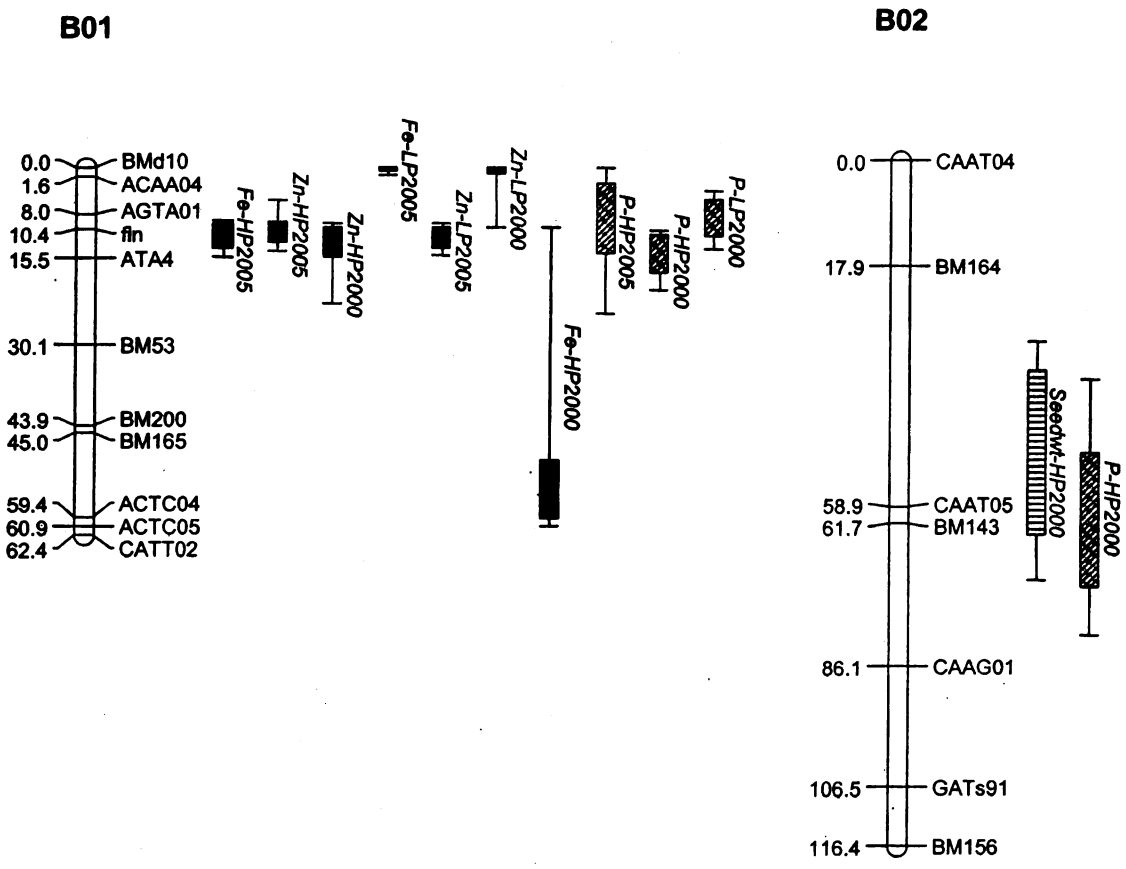


Figure 4. AND696/G19833 linkage map with QTL locations for seed P, Fe, and Zn concentration, percent phytic acid in the seed (PA), and seed weight (seedwt). QTL are further identified by treatment, high P (HP) and low P (LP) and year, 2000 and 2005.

Figure 4 (cont'd).

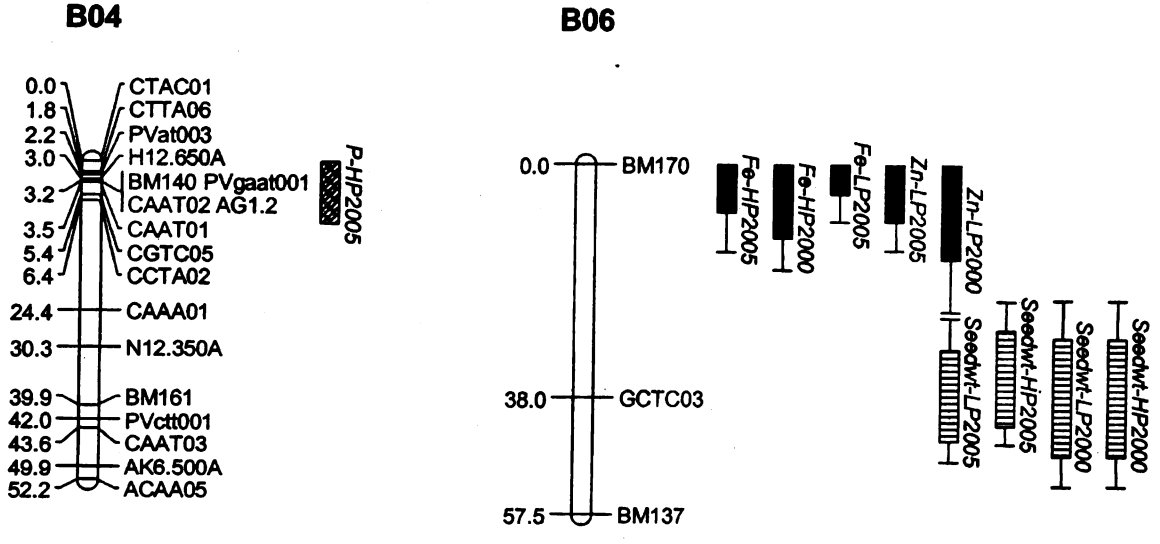
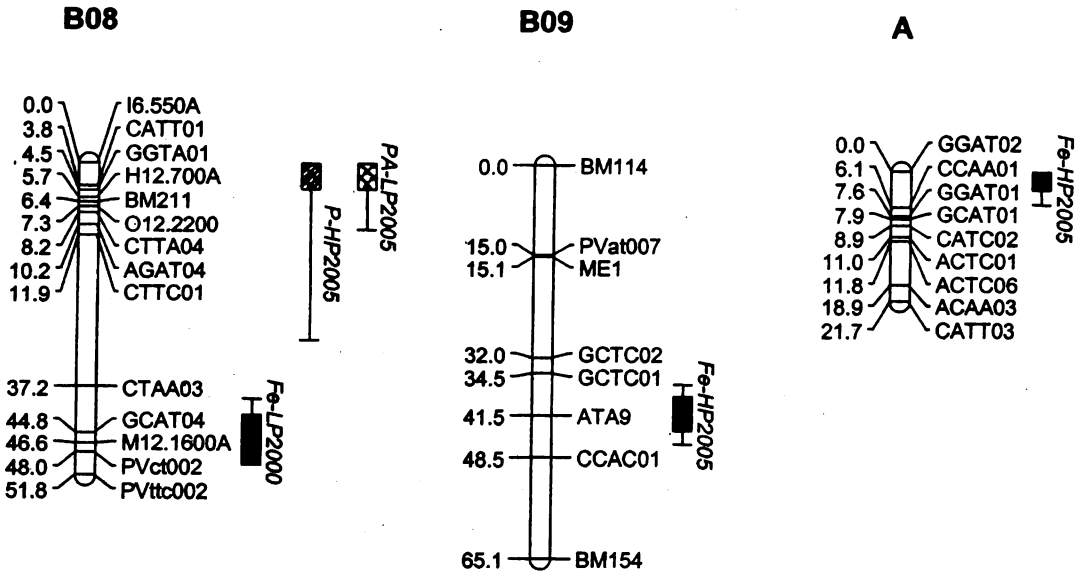


Figure 4 (cont'd).



APPENDIX

Table A2-1. Mean response for root dry weight, root length, and average root diameter in the top soil layer of different graft combinations of common bean lines FR266 and Montcalm grown in containers with a layer of compacted soil in the presence (+ Fus) or absence (-Fus) of *Fusarium solani* f.sp *phaseoli* inoculum, Data analyzed across 1 run (Nov. 2005) of experiment 2.

Top Soil Layer		**Root dry weight (mg)**		**Root length (cm)**		**Average root diameter (mm)**	
Graft combination							
Root	**Shoot**	**-Fus**	**+Fus**	**-Fus**	**+Fus**	**-Fus**	**+ Fus**
FR266	ungrafted	155	158	1088	676	0.41	0.43
FR266	FR266	84	89	765	461	0.40	0.44
FR266	Montcalm	81	84	660	326	0.41	0.46
Montcalm	ungrafted	107	99	1050	525	0.37	0.42
Montcalm	Montcalm	84	78	989	436	0.39	0.46
Montcalm	FR266	88	50	965	449	0.39	0.39
LSD 0.05†		17		182		0.02	

ANOVA

Source	**df**		**p value**	
Replication	**3**	0.4575	0.1083	0.9271
Graft (G)	**5**	<0.0001	0.1899	0.0057
Fusarium (F)	**1**	0.4369	<0.0001	<0.0001
G*F	**5**	0.6759	0.9385	0.1356

† LSD value to compare any values within root dry weight, root length, and average root diameter variables.

Table A2-2. Mean response for root dry weight, root length, and average root diameter in the middle soil layer of different graft combinations of common bean lines FR266 and Montcalm grown in containers with a layer of compacted soil in the presence (+ Fus) or absence (-Fus) of *Fusarium solani* f.sp *phaseoli* inoculum, Data analyzed across 1 run (Nov. 2005) of experiment 2.

Middle (compacted) Soil Layer		**Root dry weight (mg)**		**Root length (cm)**		**Average root diameter (mm)**	
Graft combination							
Root	**Shoot**	**-Fus**	**+Fus**	**-Fus**	**+Fus**	**-Fus**	**+ Fus**
FR266	ungrafted	20	24	247	215	0.38	0.37
FR266	FR266	4	7	123	84	0.33	0.37
FR266	Montcalm	8	9	133	94	0.39	0.37
Montcalm	ungrafted	22	15	261	180	0.37	0.37
Montcalm	Montcalm	7	5	146	40	0.38	0.37
Montcalm	FR266	4	7	100	119	0.45	0.36
LSD 0.05†		3		54		0.02	

ANOVA

Source	**df**		**p value**	
Replication	**3**	0.9691	0.7316	0.1015
Graft (G)	**5**	<0.0001	0.0089	0.1682
Fusarium (F)	**1**	0.9411	0.0947	0.2063
G*F	**5**	0.2655	0.8398	0.0487

† LSD value to compare any values within root dry weight, root length, and average root diameter variables.

Table A2-3. Mean response for root dry weight, root length, and average root diameter in the bottom soil layer of different graft combinations of common bean lines FR266 and Montcalm grown in containers with a layer of compacted soil in the presence (+ Fus) or absence (-Fus) of *Fusarium solani* f.sp *phaseoli* inoculum, Data analyzed across 1 run (Nov. 2005) of experiment 2.

Bottom Soil Layer		**Root dry weight (mg)**		**Root length (cm)**		**Average root diameter (mm)**	
Graft combination							
Root	**Shoot**	**-Fus**	**+Fus**	**-Fus**	**+Fus**	**-Fus**	**+ Fus**
FR266	ungrafted	175	216	3698	4322	0.36	0.40
FR266	FR266	41	57	1306	1204	0.35	0.36
FR266	Montcalm	69	95	1700	1667	0.35	0.36
Montcalm	ungrafted	140	141	3639	3082	0.36	0.35
Montcalm	Montcalm	38	28	1026	667	0.33	0.37
Montcalm	FR266	36	76	1554	1568	0.34	0.34
LSD 0.05†		31		347		0.01	

ANOVA

Source	**df**		**p value**	
Replication	**3**	0.9101	0.6953	0.5281
Graft (G)	**5**	<0.0001	<0.0001	0.0307
Fusarium (F)	**1**	0.2533	0.5803	0.0633
G*F	**5**	0.8542	0.3902	0.1465

† LSD value to compare any values within root dry weight, root length, and average root diameter variables.

Table A3-1. Bartlett test for variance homogeneity based on growth habit, where there were two classes, determinate and indeterminate, and 1 degree of freedom. Tests were conducted for shoot and seed traits measured under low P (LP) and high P (HP) treatments in the recombinant inbred line population developed from a cross between common bean lines AND696 and G19833.

Traits	P level	Chi-square	Pr>Chi-Square
P uptake	**HP**	1.06	0.3032
	LP	0.49	0.4851
P use efficiency	**HP**	0.01	0.9206
	LP	0.36	0.5468
Days to maturity 2005	**HP**	10.35	0.0013*
	LP	0.03	0.8631
Days to maturity 2000	**HP**	1.58	0.2081
	LP	0.06	0.8094
Seed P content 2005	**HP**	9.56	0.0020*
	LP	0.03	0.8561
Seed P content 2000	**HP**	4.98	0.0257*
	LP	2.76	0.0967
Seed yield 2005	**HP**	1.13	0.2888
	LP	1.07	0.3015
Seed yield 2000	**HP**	0.76	0.3847
	LP	6.07	0.0137*

* Variance is not homogeneous between growth habit classes

Table A3-2. Bartlett test for variance homogeneity based on growth habit, where there were two classes, determinate and indeterminate, and 1 degree of freedom. Tests were conducted for roots traits measured under low P (LP) and high P (HP) treatments in the recombinant inbred line population developed from a cross between common bean lines AND696 and G19833.

Traits	P level	Chi-square	Pr>Chi-Square
Adventitious roots	**HP**	0.12	0.7266
	LP	2.37	0.1234
Root length density	**HP**	8.62	0.0033*
	LP	1.86	0.1723
Specific root length	**HP**	16.31	<0.0001*
	LP	0.63	0.4259
Root surface area	**HP**	7.98	0.0047*
	LP	1.37	0.2418
Average root diameter	**HP**	0.21	0.6431
	LP	0.11	0.7358

* Variance is not homogeneous between growth habit classes

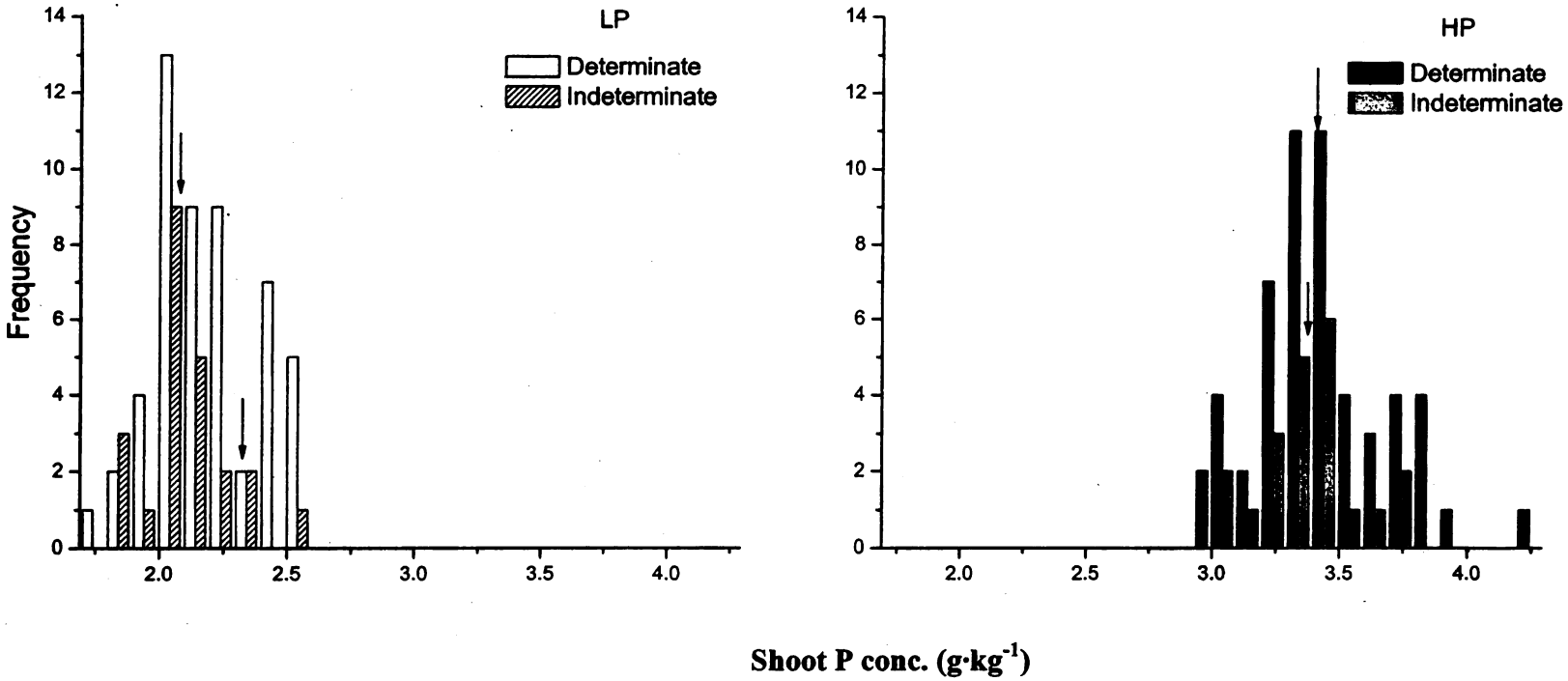


Figure A3-1. Frequency distribution of shoot P concentration, P uptake, and P use efficiency of 75 recombinant inbred lines (RILs) developed from a cross between AND696 and G19833 planted in Darien, Colombia in 2005 in high (HP) and low (LP) phosphorus soil. The distribution of RILs in LP is shown on the graphs on the left and on graphs on the right show distribution under HP. Frequency distributions are separated by soil P level with LP represented by graphs on the left and HP by graphs on the right. Within each graph RILs are also separated by growth habit. Arrows represent mean values of determinate and indeterminate RILs within each treatment. Means are the average of 3 replications.

Figure A3-1 (cont'd).

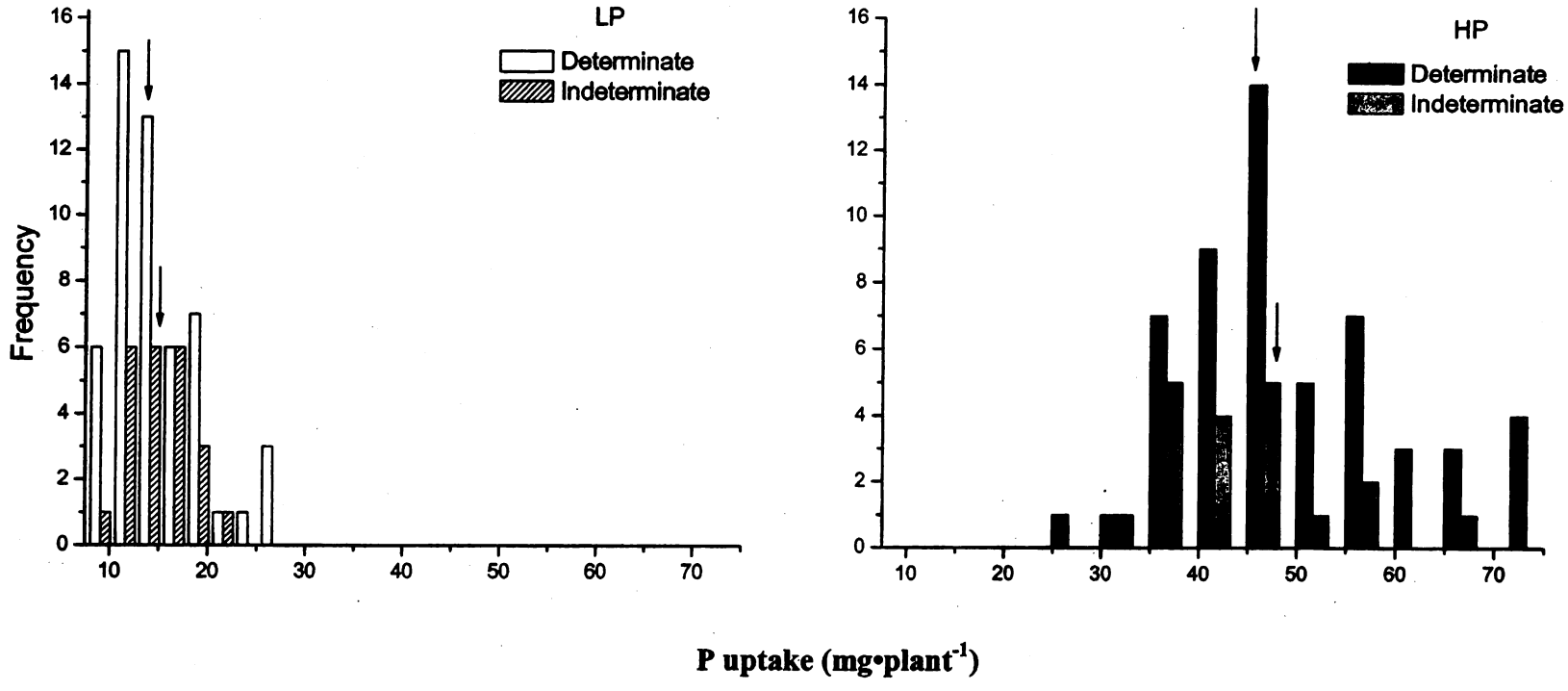
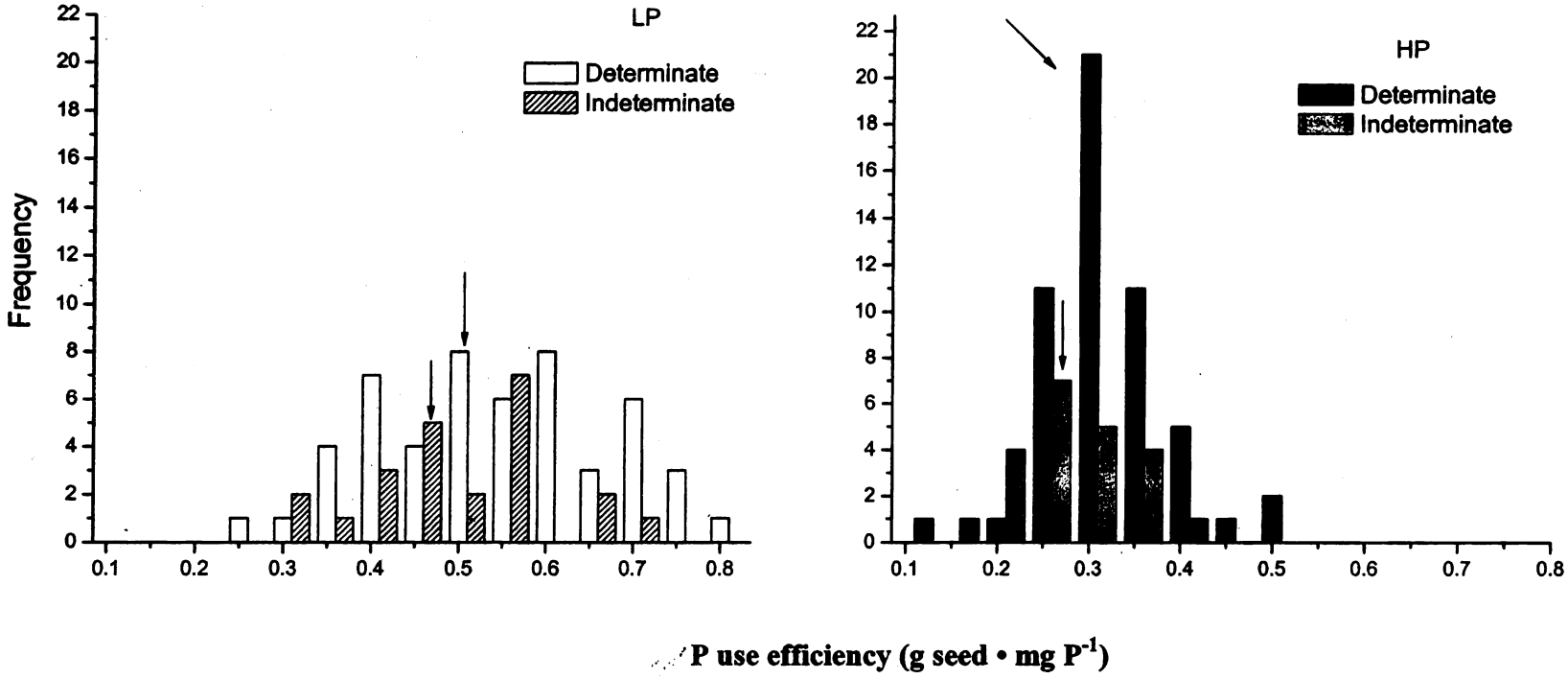


Figure A3-1 (cont'd).



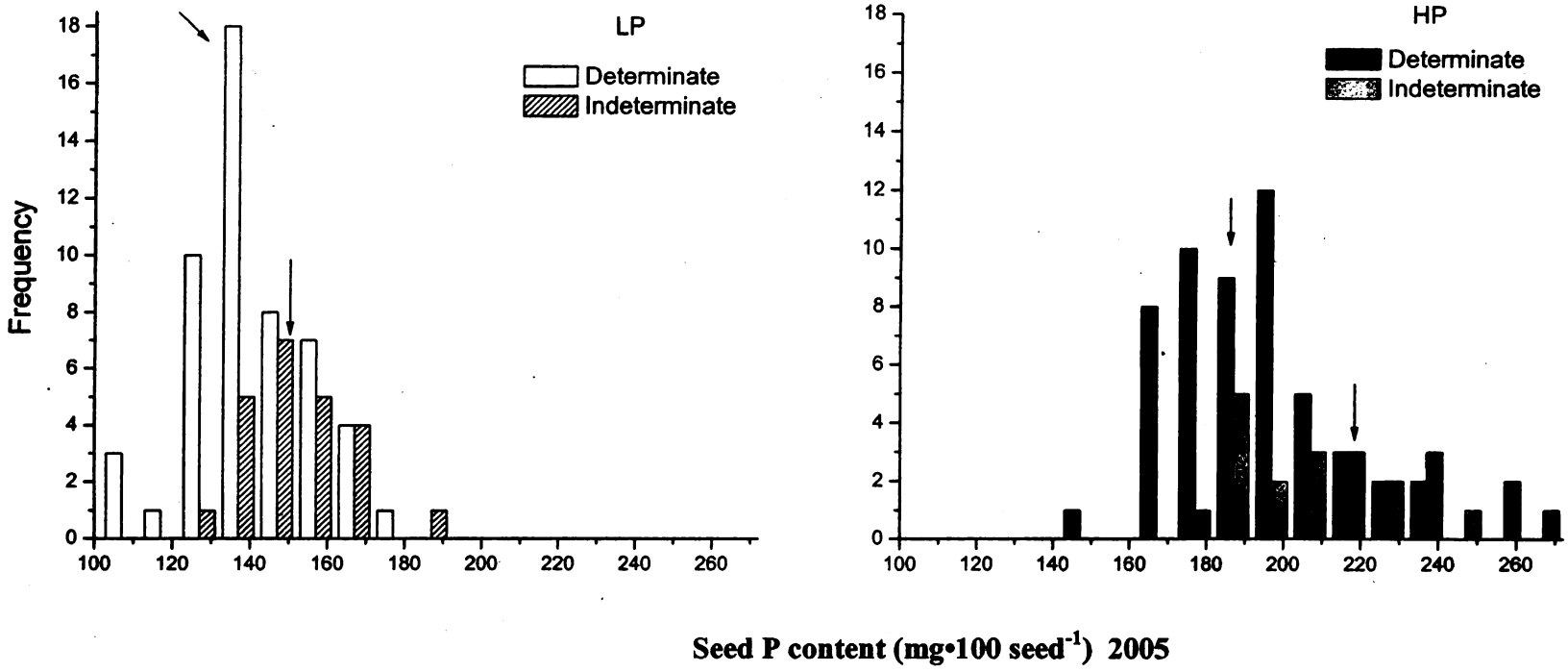
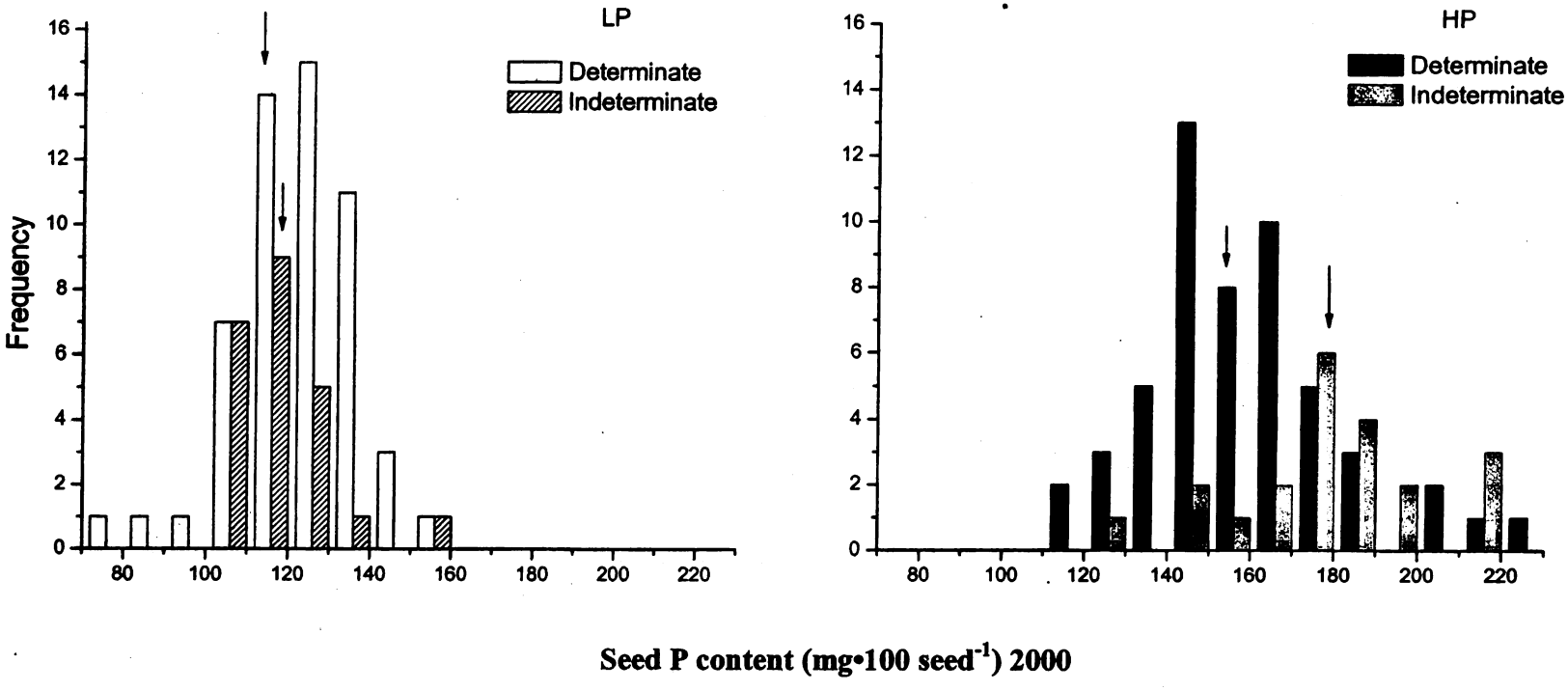


Figure A3-2. Frequency distribution of seed P content in 75 recombinant inbred lines (RILs) developed from a cross between AND696 and G19833 planted in Darien, Colombia in 2000 and 2005 in high (HP) and low (LP) phosphorus soil. The distribution of RILs in LP is shown on the graphs on the left and on graphs on the right show distribution under HP. Frequency distributions are separated by soil P level with LP represented by graphs on the left and HP by graphs on the right. Within each graph RILs are also separated by growth habit. Arrows represent mean values of determinate and indeterminate RILs within each treatment. Means are the average of 3 replications.

Figure A3-2 (cont'd).



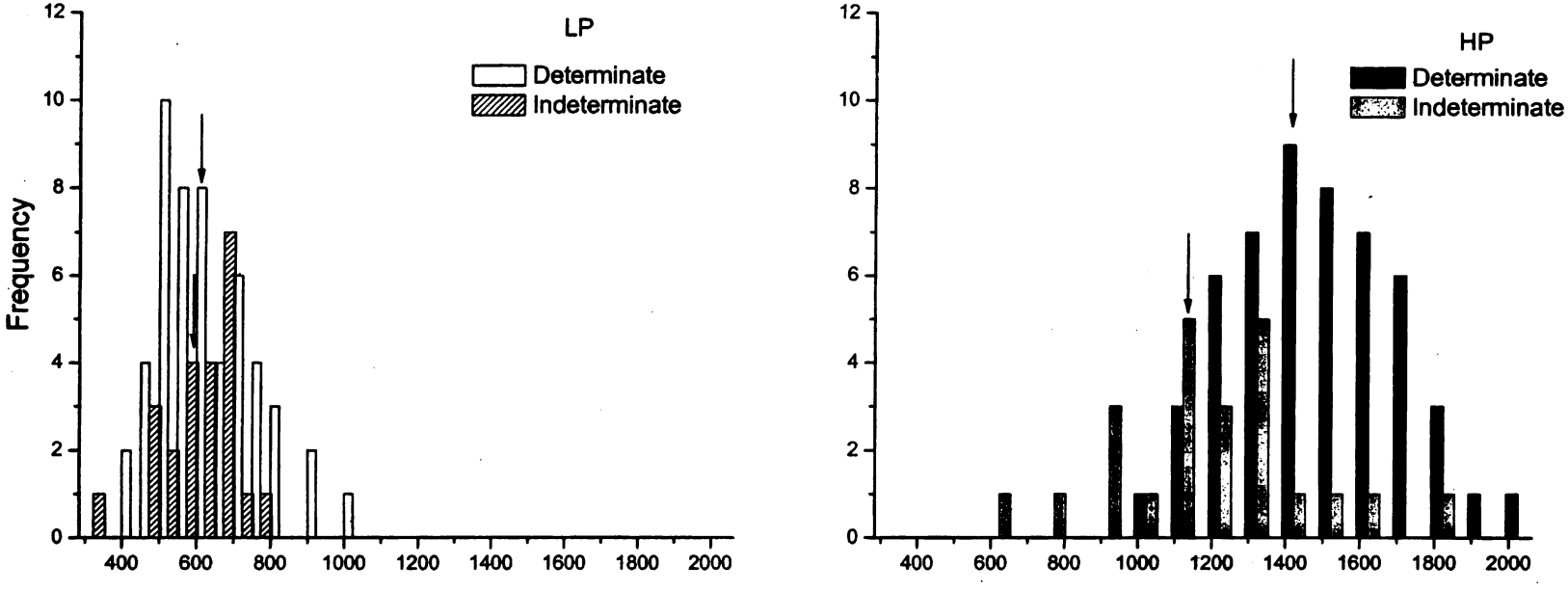


Figure A3-3. Frequency distribution of seed yield in 75 recombinant inbred lines (RILs) developed from a cross between AND696 and G19833 planted in Darien, Colombia in 2000 and 2005 in high (HP) and low (LP) phosphorus soil. The distribution of RILs in LP is shown on the graphs on the left and on graphs on the right show distribution under HP. Frequency distributions are separated by soil P level with LP represented by graphs on the left and HP by graphs on the right. Within each graph RILs are also separated by growth habit. Arrows represent mean values of determinate and indeterminate RILs within each treatment. Means are the average of 3 replications.

Figure A3-3 (cont'd).

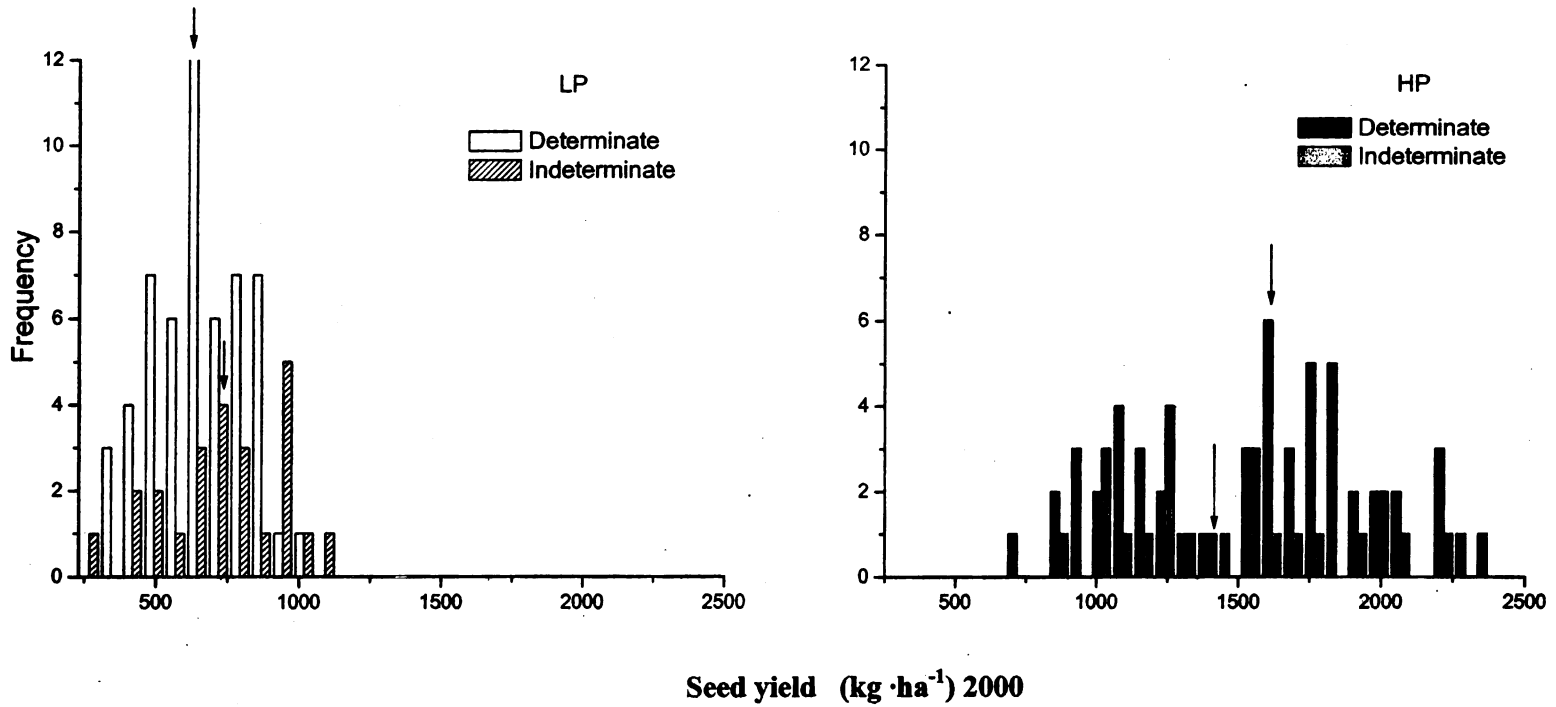


Table A4-1. Amplified fragment length polymorphisms (AFLP) names and selective primers used in developing a linkage map of AND696/G19833.

AFLP name	Selective primer 1[†]	Selective primer 2[‡]	Polymorphism number[*]
ACAA	E-AAC	M-CAA	5
ACAC	E-AAC	M-CAC	4
ACAT	E-AAC	M-CAT	3
ACTA	E-AAC	M-CTA	2
ACTC	E-AAC	M-CTC	6
ACTG	E-AAC	M-CTG	3
ACTT	E-AAC	M-CTT	2
AGAC	E-AAG	M-CAC	1
AGAT	E-AAG	M-CAT	6
AGTA	E-AAG	M-CTA	2
AGTC	E-AAG	M-CTT	3
CAAA	E-ACA	M-CAA	3
CAAC	E-ACA	M-CAC	5
CAAG	E-ACA	M-CAG	1
CAAT	E-ACA	M-CAT	5
CATC	E-ACA	M-CTC	2
CATT	E-ACA	M-CTT	3
CCAA	E-ACC	M-CAA	3
CCAC	E-ACC	M-CAC	1
CCTA	E-ACC	M-CTA	2
CCTT	E-ACC	M-CTT	1
CGAA	E-ACG	M-CAA	3
CGAC	E-ACG	M-CAC	1
CGTA	E-ACG	M-CTA	3
CGTC	E-ACG	M-CTC	5
CGTT	E-ACG	M-CTT	1
CTAA	E-ACT	M-CAA	4
CTAC	E-ACT	M-CAC	1
CTTA	E-ACT	M-CTA	6
CTTC	E-ACT	M-CTC	1
CTTT	E-ACT	M-CTT	1
GCAC	E-AGC	M-CAC	1
GCAT	E-AGC	M-CAT	5
GCTC	E-AGC	M-CTC	4
GGAA	E-AGG	M-CAA	3
GGAG	E-AGG	M-CAG	2
GGAT	E-AGG	M-CAT	3
GGTA	E-AGG	M-CTA	3

[†]EcoR1 adapter (E) ligated to 3 selective nucleotides (i.e. AAC).
[‡] Mse1 adapter (M) ligated to 3 selective nucleotides (i.e. CAA).
[*]in AND696/G19833 population with selective primer combination 1 and 2.

Table A4-2. Simple sequence repeat (SSR) primer sequence, linkage group and source information for markers polymorphic between AND696 and G19833 and used to develop a linkage map of AND696/G19833.

SSR	Primer†	Primer Sequence	Linkage group‡	Source*
AG1	F	CAT GCA GAG GAA GCA GAG TG	B03	2
	R	GAG CGT CGT CGT TTC GAT		
ATA13	F	unpublished	B03	5
	R			
ATA4	F	unpublished	B01	5
	R			
ATA9	F	unpublished	B09	5
	R			
BM114	F	AGC CTG GTG AAA TGC TCA TAG	B09 (unlinked)	2
	R	CAT GCT TGT TGC CTA ACT CTC T		
BM137	F	CCG TAT CCG AGC ACC GTA AC	B06	2
	R	CGC TTA CTC ACT GTA CGC ACG		
BM140	F	TGC ACA ACA CAC ATT TAG TGA C	B04	2
	R	CCT ACC AAG ATT GAT TTA TGG G		
BM143	F	GGG AAA TGA ACA GAG GAA A	B02	2
	R	ATG TTG GGA ACT TTT AGT GTG		
BM154	F	TCT TGC GAC CGA GCT TCT CC	B09	2
	R	CTG AAT CTG GGA ACG ATG ACC AG		
BM156	F	CTT GTT CCA CCT CCC ATC ATA GC	B02	2
	R	TGC TTG CAT CTC AGC CAG AAT C		
BM159	F	GGT GCT GTT GCT GCT GTT AT	B03	2
	R	GGG AGA TGT GGT AAG ATA ATG AAA		

Table A4-2 (cont'd).

SSR	Primer†	Primer Sequence	Linkage group‡	Source*
BM160	F	CGT GCT TGG CGA ATA GCT TTG	B07	2
	R	CGC GGT TCT GAT CGT GAC TTC		
BM161	F	TGC AAA GGG TTG AAA GTT GAG AG	B04	2
	R	TTC CAA TGC ACC AGA CAT TCC		
BM164	F	CCA CCA CAA GGA GAA GCA AC	B02	2
	R	ACC ATT CAG GCC GAT ACT CC		
BM165	F	TCA AAT CCC ACA CAT GAT CG	unlinked (B01)	2
	R	TTC TTT CAT TCA TAT TAT TCC GTT CA		
BM167	F	TCC TCA ATA CTA CAT CGT GTG ACC	B02 (unlinked)	2
	R	CCT GGT GTA ACC CTC GTA ACA G		
BM170	F	AGC CAG GTG CAA GAC CTT AG	B06	2
	R	AGA TAG GGA GCT GGT GGT AGC		
BM200	F	TGG TGG TTG TTA TGG GAG AAG	B01	2
	R	ATT TGT CTC TGT CTA TTC CCA C		
BM211	F	ATA CCC ACA TGC ACA AGT TTG G	B08	2
	R	CCA CCA TGT GCT CAT GAA GAT		
BM53	F	AAC TAA CCT CAT ACG ACA TGA AA	B01	2
	R	AAT GCT TGC ACT AGG GAG TT		
BMd-1	F	CAA ATC GCA ACA CCT CAC AA	B03 (unlinked)	3
	R	GTC GGA GCC ATC ATC TGT TT		

Table A4-2 (cont'd).

SSR	Primer†	Primer Sequence	Linkage group‡	Source*
BMd-10	F	GCT CAC GTA CGA GTT GAA TCT CAG	B01	3
	R	ATC TGA GAG CAG CGA CAT GGT AG		
BMd-3	F	TGT TTC TTC CTT ATG GTT AGG TTG	unlinked (B07)	3
	R	GTA TCC TCC GAT CAA ATT CAC CT		
BMd-42	F	TCA TAG AAG ATT TGT GGA AGC A	B10	3
	R	TGA GAC ACG TAC GAG GCT GTA T		
Gats91	F	GAG TGC GGA AGC GAG TAG AG	B02	2
	R	TCC GTG TTC CTC TGT CTG TG		
ME1	F	unpublished	B09	5
	R			
PVct002	F	TTA GAC TTT CAA ACA TTC AC	B08	4
	R	GAT ACT ACT TAA ATG AGG AAC A		
PVttc002	F	ATA TCT TAC AGC CAT TAC ATT C	B08	4
	R	CTC ATC ACC CAG TCA CCT		
PVctt001	F	CCA ACC ACA TTC TTC CCT ACG TC	B04	1
	R	GCG AGG CAG TTA TCT TTA GGA GTG		
PVag001	F	CAA TCC TCT CTC TCT CAT TTC CAA TC	B11	1
	R	GAC CTT GAA GTC GGT GTC GTT T		
PVat003	F	ACC TAG AGC CTA ATC CTT CTG CGT	B04	1
	R			
PVgaat001	F	AAG GAT GGG TTC CGT GCT TG	B04	1
	R	CAC GGT ACA CGA AAC CAT GCT ATC		

Table A4-2 (cont'd).

SSR	Primer†	Primer Sequence	Linkage group‡	Source*
PVat007	F	AGT TAA ATT ATA CGA GGT TAG CCT AAA TC	B09	1
	R	CAT TCC CTT CAC ACA TTC ACC G		

† Primer orientation: forward (F); reverse (R)
‡Bean linkage group location of SSR in the consensus map (Freyre et al., 1998), if different than where it mapped to in AND696/G19833 population, that location is noted in parenthesis.
* 1 :Yu et al., 2000. Journal of Heredity 91(6):429-434.
2 : Gaitan-Solis et al., 2002. Crop Science 42:2128-2136.
3 : Blair et al. 2003. Theoretical Applied Genetics 107:1362-1374.
4 : Caixeta et al., 2005. Crop Breeding and Applied Biotechnology 5:125-133.
5: Metais et al., 2002. Theoretical Applied Genetics 104:1346-1352.
(full citations available in Chapter 4, References)

Table A4-3. Random Amplified Polymorphism DNA (RAPD) primer sequence for markers polymorphic between AND696 and G19833 and used to develop a linkage map of AND696/G19833 recombinant inbred line population.

RAPD name	**Primer sequence**	**Polymorphism number†**
AA-19	5'-TGAGGCGTGT-3'	1
AK-6	5'-TCACGTCCCT-3'	2
H-12	5'-ACGCGCATGT-3'	2
L-4	5'-GACTGCACAC-3'	1
I-6	5'-AAGGCGGCAG-3'	1
M-2	5'-ACAACGCCTC-3'	1
N-12	5'-CACAGACACC-3'	1
R-4	5'-CCCGTAGCAC-3'	1
O-12	5'-CAGTGCTGTG-3'	2

†Number of polymorphisms with this primer in the AND696/G19833 recombinant inbred line population.

Table A4-4. Bartlett test for variance homogeneity based on growth habit, where there were two classes, determinate and indeterminate, and 1 degree of freedom. Tests were conducted for seed traits measured under low P (LP) and high P (HP) treatments in the recombinant inbred line population developed from a cross between common bean lines AND696 and G19833.

Traits	P level	Chi-square	Pr>Chi-Square
		2005	
Seed weight	**HP**	13.25	0.0003*
	LP	1.02	0.3135
Seed P	**HP**	2.66	0.1029
	LP	1.17	0.2799
Seed Fe	**HP**	1.45	0.2290
	LP	1.51	0.2190
Seed Zn	**HP**	1.47	0.2261
	LP	12.60	0.0004*
Seed phytic acid	**HP**	0.55	0.4598
	LP	22.95	<0.0001*
		2000	
Seed weight	**HP**	1.57	0.2095
	LP	0.07	0.7903
Seed P	**HP**	6.44	0.0112*
	LP	0.31	0.5781
Seed Fe	**HP**	0.06	0.8004
	LP	0.88	0.3484
Seed Zn	**HP**	0.96	0.3260
	LP	1.00	0.3173

* Variance is not homogeneous between growth habit classes