CHARACTERIZATION OF DIVERSE SOYBEAN GENOTYPES FOR PHOSPHORUS UPTAKE AND USE EFFICIENCY

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DEDICATION

I would like to dedicate this dissertation to my family whose love, encouragement, and support throughout my PhD. study and life endeavors. To my parents, Mr. Bao D. Nguyen and Mrs. Diep T. Nguyen, who raised me to be kind and perseverance. To my children, Anh D. Dao, Ha T. Dao, and David Dao, who always make me happy and ensure my life does not completely revolve around research.

"Never to forget where we came from and always praise the bridges that carried us over"

-Fannie Lou Hamer

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With regardful memories…

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BM	Biomass
BRC	Bradford research center
DNA	Deoxyribonucleic acid
EI	Efficient index
ENR	Efficient non-response
ER	Efficient response
INR	Inefficient non-response
IR	Inefficient response
MAP	Mono ammonium phosphate
NAM	Nested association mapping
P	Phosphorus
PAE	Phosphorus acquisition efficiency
PI	Plant introduction
PUE	Phosphorus use efficiency
QTL	Quantitative trait locus
RCBD	Random complex block design
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RPAE	Root phosphorus acquisition efficiency
RSR	Root to shoot ratio
RW	Root weight
SLAF	Specific length amplified fragment
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeats
TLA	Total leaf area

ABSTRACT

Soybean (*Glycine max* L. Merrill) is one of the most important legume crops grown worldwide. However, without phosphorus (P) fertilization, soybean yields often are limited by phosphorus availability. Phosphorus is an essential macronutrient and P uptake ability and P use efficiency (PUE) of a crop critically influence its productivity. To improve soybean yields under low-P conditions, a better understanding of the mechanisms underlying P uptake and PUE is needed. The first part of this study was aimed at identifying and characterizing soybean genotypes which contrast in their ability to take up P and in their PUE. Results from pot and field experiments with diverse soybean genotypes, including SoyNAM parents, obsolete cultivars, commercial cultivars, and plant introduction lines, revealed significant differences among genotypes for numerous P-related traits. Significant differences in shoot P concentration, shoot P content used as surrogate measure for P uptake, physiological PUE, and root complexity were observed among genotypes. Phosphorus use efficiency was much higher under low-P conditions compared to P-sufficient conditions. Positive correlations between biomass production and P uptake and top-soil root architecture and P uptake were observed. In a subset of five contrasting genotypes, soybean root symbiosis with arbuscular mycorrhizal fungi (AMF) was investigated to explore whether mycorrhizal infection levels were related with genotype differences in P uptake and PUE. All five genotypes displayed high AMF colonization percentages (> 80%) and no significant differences in mycorrhizal colonization were detected among genotypes and between low-P and P sufficient treatments. Arbuscular mycorrhizal fungi colonization did not explain observed differences in P uptake, and approaches aimed at increasing levels of AMF infection in soybean do not

appear promising, at least not for environments like the one used in this study. This research identified soybean genotypes contrasting for shoot P concentration, shoot P content, PUE, and topsoil root system architecture. Further, it confirmed differential sensitivity of diverse soybean genotypes to P availability. The identified genotypes can serve as a resource for physiological and genetic studies as well as in breeding efforts aimed at improving P uptake and PUE in elite germplasm.

CHAPTER 1

LITERATURE REVIEW

1. Introduction

Soybean (*Glycine max* L. Merrill) is a valuable crop because of its multiple uses, including as an oil seed crop and as a source of protein. At present, soybean occupies an area of approximately 131.89 million hectares, producing 385.14 million metric tons with an average productivity of 2.92 metric tons per hectare globally. In the United States of America, it occupies an area of 34.98 million hectares with a production of 121.06 million metric tons in 2020/2021 and an average productivity of 3.46 metric tons per hectare (Anonymous, 2021).

Soybean is a legume and has been adapted to diverse climatic conditions from tropical and subtropical to temperate climates. Soybean is important for its high protein content (about 40%), oil content (20%), and excellent amounts of dietary fiber, vitamins, and minerals (Liu, 1999). Soybean has garnered global interest not only because of its nutritive value but also due to its ability to improve soil fertility through symbiotic association with the N-fixing bacterium *Bradyrhizobium japonicum*. However, like other crops, the production of soybean can be severely limited by low phosphorus (P) availability.

Phosphorus is one of the key components for plants and is essential for plant growth and development as it is a constituent of various cellular components, including nucleic acids (DNA, RNA), cell membranes (phospholipids), and the energy currency ATP. Under P deficiency, plants are stunted and more disease susceptible.

As the world population increases, the need for food production increases which leads to increased demand for P fertilizer (Cordell and White, 2014). However, the access to P, mainly from rock phosphate, is limited and highly concentrated. The production of P fertilizer from this nonrenewable resource can only be found in a relatively small number of countries, including Morocco, China, Jordan, South Africa, and the USA (Van Kauwenbergh, 2010; Jasinski, 2011). It is estimated that the annual P production will reach a maximum by 2033 and then fall below the demand for agriculture (Cordell and White, 2011). This becomes a big concern for food security globally.

Extensive use of P fertilization has led to negative environmental impacts such as eutrophication. Of the P fertilizer applied, about 80% is fixed in the soil and some is lost to the environment through run off. This P accumulates in water bodies and is a major reason for algal bloom which leads to oxygen depletion which results in animal death and so-called dead-zones. The excess of P in water bodies therefore impacts the ecosystem as well as local economies (Carvalho *et al.,* 2013). Development of crops with superior ability to take up P from the soil and that can use the acquired P more efficiently can help address both the issues of limited P reserves as well as negative environmental impacts from excessive application of P fertilizers.

2. Phosphorus and plants

2.1. The importance of phosphorus

Phosphorus is an essential macronutrient that limits crop productivity, including that of soybean, in many environments. Phosphorus is known as “the key to life” because it is a constituent of molecules such as nucleic acids in the cell nucleus: deoxyribonucleic

acid (DNA) and ribonucleic acid (RNA), phospholipids in cell membranes, and adenosine triphosphate (ATP) (Hewitt, 1963; Marschner, 1995; Schachtman *et al*., 1998; Fageria, 2009; Fageria *et al*., 2013). As such, it is involved in critical processes including photosynthesis, energy transfer, DNA replication, and nitrogen fixation (Hermandez *et al.*, 2009; Malhotra *et al*., 2018). Additionally, in soybean, P is needed for nodule development and functioning (Sa and Israel, 1991). Phosphorus also is necessary for processes such as tillering, branching, and root development, and seed formation.

2.2. Phosphorus deficiency symptoms

While largely immobile in soil, P is mobile in the plant (phloem), which causes P deficiency symptoms to first appear in older leaves (Marschner, 1995). For optimum growth, at vegetative stages, plants require a P tissue concentration of about 0.3 to 0.5%. Under low soil P conditions, plants tend to exhibit reduced leaf expansion, auxiliary bud growth, and carbohydrate utilization, but increased soluble protein and chlorophyll content per unit leaf area, resulting in small and dark green leaves. Sometimes, red, purple, or brown pigments also appear on leaves, especially along veins, depending on the severity of P deficiency (White and Hammond, 2008). Phosphorus deficiency causes reductions in cell division rates and cell expansion, leading to smaller plants. In P deficient plants, reduced photosynthesis, respiration, and abundance of C, N and S metabolites, as well as altered plant hormone regulation cause stunted growth (Marschner, 1995). Severe P deficiency can cause chloroplast abnormalities by reducing the number and changing the morphology of grana which adversely affects chloroplast function (White and Hammond, 2008). Phosphorus availability also alters the shoot-root ratio of plants with P deficient plants growing

relatively more roots than shoots to improve P acquisition (Lynch *et al*., 1991; Marschner *et al*., 1996; Rao and Terry, 1995; Nielsen *et al*., 1998; Nielsen *et al*., 2001).

3. Phosphorus in soil

3.1. Form of phosphorus in soil

Phosphorus is abundant in soil but mostly fixed or bound to clay particles which renders it unavailable for plant uptake. Research shows that about 80 to 90% of applied P fertilizer is fixed and only 10 to 20 % is available for plant uptake (Gerke *et al*., 1994; Jones, 1998). Phosphorus in soil occurs in solution ($H_2PO_4^-$ and HPO_4^{2-}), as inorganic soil P, and as organic P. The form and availability of P in soil depends on many factors and processes, including pH, precipitation, adsorption and desorption, and mineralization and immobilization. In acid soil (pH< 7), P is precipitated by Al and Fe or is adsorbed to Al and/or Fe oxide and clay minerals. In alkaline soil (pH>7), P is adsorbed to Ca carbonate and clay minerals and/or precipitated as minerals of Ca-P and Mg-P. When these precipitates are dissolved, P is released and becomes available for plant uptake. Plants take up dihydrogen phosphate ($H_2PO_4^-$) and mono-hydrogen phosphate (HPO_4^{2-}).

Mineralization and immobilization occur due to the presence of microorganisms in soil and convert organic P to inorganic P and *vice versa*. Mineralization makes the P available for plants by decomposition (oxidation) of the chemical compounds in organic matter into inorganic forms which can be taken up by plants. In contrast, uptake, and utilization of available P by microorganisms (immobilization) makes P unavailable to plants. The amount of organic P mineralized in the soil is related to the amount of organic matter present in the soil and the release of P from organic matter is mediated by the action

of phosphatase enzymes, including some that can be released by plants. Indeed, plant growth promoting rhizobacteria are well known for their beneficial effects on plant growth by enhancing P availability through solubilization of precipitated inorganic P and mineralization of organic P (Kang *et al*., 2002; Pradhan and Sukala, 2005; Chen, *et al*., 2006).

Phosphorus availability to plants also is limited by other processes such as run off, erosion and leaching. Run off is the main process that causes P pollution of water bodies; it is influenced by factors such as the slope of a field, amounts of residue on the soil, tillage practices, and plant growth and cover.

3.2. Interactions of phosphorus with other nutrients in soil

Proper growth and development of plants requires availability of sufficient essential mineral nutrients. The general interaction of P with other nutrients affects plant health, yield, and pathogen infections (Hopkins, 2015). Phosphorous is one of 17 essential nutrients and is in particularly high demand by fast growing crops such as potatoes and vegetables (Nishomoto *et al.*, 1977; Greenwood *et al*., 1980; Itoh and Barber, 1983; Alt, 1987; Sanchez *et al*., 1990). Plants absorb nutrient elements primarily as ions dissolved in water. Thus, interactions among elements in the soil and soil solution will affect plant performance and crop yield.

The influence of nutrient interactions on crop growth can be negative (Antagonism), no interaction (Factors additive), or positive (Synergism) (Summer and Farina, 1986). This is critical as excessive application of one nutrient can induce a deficiency of another. For instance, too much P in soils induces Cu, Fe, Zn, and Bo

deficiency and reduces availability of K, whereas incorporation of appropriate amounts of P and Fe can increase the uptake of both nutrients (Black, 2019).

In terms of P, N fertilizer application increases P availability and uptake by plants. Both NO_3^- and NH_4^+ enhance the uptake of P. Positive relationships of P availability on S and Mg uptake also have been documented (Rietra *et al*., 2017). The most common interaction of P with micronutrients is antagonism (Brown and Tiffin, 1962). Antagonistic interaction happens when excessive application of one nutrient causes reduced uptake of the other nutrient. For example, excessive amounts of P reduce uptake of cationic micronutrients like Fe, Mn, Zn and Cu. Zinc and P interaction is the most commonly observed antagonistic interaction. Plants absorb P as anions ($H_2PO_4^{-1}$ or HPO_4^{-2}) and Zn as a cation (Zn^{2+}). These positively and negatively charged ions attract one another to create a strong P-Zn bond. Excessive application of P leads to decrease of Zn absorption in potato (Soltanpour, 1969; Christensen, 1972; Christensen and Jackson, 1981). Similar results were observed in soybean, corn, wheat, rice, and groundnut, (Adriano *et al*., 1971; Barker, 1978; Haldar and Mandal, 1981; Sharma *et al*., 1986; Webb and Loneragan, 1988; Nayak and Gupta, 1995; Adriano, 2001; Bukovic *et al*., 2003; Mirvat *et al*., 2006; Shittu and Ogunwale, 2012).

3.3. Phosphorus fertilizer use in agriculture and its impact on environments

Unlike N, P is only available in soil and is taken up by plants in the orthophosphate form through the root systems. Phosphorus is abundant in soil, but it is mostly in insoluble forms or being fixed as Fe and Al phosphates in acidic soils, and Ca phosphates in high pH soil such that application of P fertilizer often is required to enhance plant growth. The use

of P fertilizers has raised concerns as the mining of rock phosphate is neither eco-friendly nor sustainable in the long term, and excessive application of P leads to eutrophication.

The main source of P is rock phosphate which is a non-renewable resource. Most of agricultural P fertilizers are manufactured from rock phosphate which has taken 10 – 15 million years to form from seabed to soil via tectonic uplift and weathering. This resource mainly is distributed in Morocco, China, and the USA. It is very rare in many areas of the world, such as Europe or West and Central Africa. Unlike the other non-renewable resources such as oil, fossil fuels (coal, petroleum, and natural gas) and nuclear energy which can be substituted with other sources like wind, biomass or thermal energy, P has no substitute in crop production (Cordell and White, 2014). It is estimated that the rock phosphate reserve will be depleted in about 500 – 600 years (Sharma *et al.*, 2013). Furthermore, this finite resource is used inefficiently in agriculture. About 80% of P fertilizers applied is fixed in the soil or lost to water bodies where it causes environmental problems such as eutrophication (Cordell and White, 2014). Environmental P pollution is caused by the processes of run off, erosion and leaching which lead to increases in P concentrations in water bodies. Phosphorus in rivers, ponds, lakes, and oceans cause algal blooms which change the pH and oxygen levels in the water leading to dead zones. Efforts to limit P losses from fields and negative environmental impacts are essential and under way on various fronts, including the development and use of polymer coated fertilizer, banding of fertilizer, maintenance of soil pH in a suitable range (~6.3 to 7), planting of cover crops, reduced tillage or conservation tillage, plant residue management, and development of crop plants with enhanced capacity to take up and utilize P.

4. Strategy of plants to overcome P deficiency

4.1. Mechanism of phosphorus uptake

Phosphorus can move from soil to plant roots by diffusion or mass flow or can be intercepted by growing roots. Since P is relatively immobile in soil, mass flow does not play a prominent role for P movement to the roots.

Monohydrophosphate and dihydrophosphate largely arrive at the root due to diffusion along the concentration gradient, and in plants can transported apoplastically or symplastically. The rate of diffusion of P in soil is very slow and depends on many factors such as soil water content, temperature, P concentration, tortuosity, soil buffering capacity and compaction as well as other nutrients.

Like other substances/solutes (ions, metabolites), the movement of P in or out of cells is driven by electrical and concentration gradients. Plants take up P in the form of orthophosphates (HPO_4^{-2} or $H_2PO_4^{-}$) that are negatively charged. Thus, the movement of P into the cell is against the electrical gradient of the interior cell (~−100 mV). The concentration of inorganic P (Pi) in soil solution typically is 1 to 10 μM, while Pi concentration in the cytoplasm is about 10 mM, which is 1,000 to 10,000 times higher. Therefore, the transport of P from the soil solution into the cell is an active transport which requires energy. This process is mediated by the proton pump ATPases (H^+-ATPases).

Specific transport proteins located in the plasma membrane are responsible for P uptake, including PHT1 and PHO1. PHT1 belongs to the family of phosphate H^+ symporters (PHS) within the major facilitator super family (MFS). PHT1 transports phosphate across the plasma membrane in response to the chemiosmotic gradient and

requires two to four protons for each phosphate. This cotransport of protons into root cells underlies the increase in pH of the extracellular medium associated with P uptake. The PHO1 proteins do not belong to the MFS but to the SPX-EXS protein family. They drive the transport of phosphate from cell to cell and require energy from ATP.

In soybean, 14 phosphate transporters have been characterized (GmPT1 – GmPT14). Among them, 12 of 14 GmPTs are high-affinity phosphate transporters, most of them involve in synergistic regulation of mineral nutrient homeostasis in soybean (Qin *et al*., 2012). Besides, GmPT5 and GmPT7 are high affinity phosphate transporters localized in the plasma membrane and involved in transport of phosphate from root cells to nodules of soybean and then translocate phosphate into the nitrogen fixation zone where phosphate is needed for biological nitrogen fixation and bacteria development (Chen *et al*., 2018).

Under P starvation conditions, plants express mechanisms to overcome and maintain growth and development. Phosphorus taken up by roots is transported in the xylem and phloem. Phosphorus limitations are sensed in roots and shoots and alter hormonal dynamics including for auxin, ethylene, cytokinins, abscisic acid, gibberellin and strigolactones which mediate growth responses aimed at overcoming P starvation (Franco-Zorrilla *et al*., 2004; Jain *et al*., 2007; O'Rourke *et al*., 2013). Signaling of P status also involves Ca^{++}, inositol polyphosphate and reactive oxygen species (ROS). PHR proteins are phosphate starvation response proteins which are considered as a central regulator of phosphate (Pi) homeostasis in several plant species, and in soybean GmPHR25 was known as key in the P signaling network (Xue

et al., 2017). Starvation sensing and signaling events alter gene expression patterns and result in changes in root system characteristics, including changes in root architecture, increased exudate production (*e.g*. organic acids, acid phosphatase), symbiosis with mycorrhizal fungi, *etc*. to improve of P acquisition. In soybean, inadequate P availability causes poor root development, short, stunted plants, inhibited nodule growth and N_2-fixing capacity (Chaudhary *et al*., 2008).

4.2. Plant adaptations to low levels of P availability

Under low P soil conditions, plants can either increase P acquisition efficiency from the soil or improve their internal P use efficiency. These two aspects constitute the P efficiency (PE) of a plant. Numerous studies have been carried out to examine P uptake and to improve PE. The strategies for improvement of P uptake vary with plant species and genotypes. Plants can adjust morphological traits of the root system, alter exudation, modulate P transporters in the plasma membrane, as well as establish symbiotic associations with mycorrhizal fungi.

Most studies focus on root traits to enhance the exploration of the soil for P and improve plant P acquisition (Lynch, 2011). Root characteristics that are important to enhance P acquisition include shallower root growth angles of axial roots (because more P tends to be available in the topsoil), more adventitious roots, a greater number of axial roots, and greater dispersion of lateral roots or root hair length and density (Miguel *et al*., 2015). In addition, changes in root characteristics which reduce carbon cost also are important and include increased number of root cortical aerenchyma in lateral roots (Postma and Lynch, 2011).

5. Adaptive mechanisms

5.1. Mechanisms to increase P uptake

5.1.1. Topsoil foraging

When faced with P starvation, one of the most common changes in the root system is enhanced topsoil foraging since P generally is more abundant in the top-soil layer (Lynch and Brown, 2001). A number of root architecture traits are involved in topsoil foraging including shallower growth angles of axial roots, enhanced adventitious rooting, and greater dispersion of lateral roots (Lynch, 2007).

5.1.2. Root hairs

Root hairs are critical for nutrient uptake and usually are found 1-2 cm from the root tip. Root hairs facilitate nutrient uptake by increasing the soil volume explored by the plant, which is especially important for nutrients with low mobility in soils, such as P (Clarkson, 1985; Peterson and Farquhar, 1996; Bates and Lynch, 2001). Indeed, longer root hairs and a higher density of root hairs have been shown to enhance P acquisition in soybean under low P conditions (Vandamme *et al*., 2013). Similarly, in maize, more root hairs are beneficial for P acquisition (Zhu *et al*., 2005). Miguel (2004) showed an advantage of root hairs for P uptake in common bean (*Phaseolus vulgaris*) even in the presence of mycorrhizal colonization. He also found a strong correlation between root hair length and root hair density among common bean genotypes. In barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*), genotypic variation in root-hair length significantly influenced P uptake from the soil (Gahoonia *et al*., 1997; Gahoonia and Nielsen, 1997). Studies with *Arabidopsis thaliana* have shown that root hair length and density were enhanced under low P availability (Bates *et al*., 1995; Bates and Lynch, 2000).

These results indicate that root hair length and density are important traits for P uptake and genetic variability of root hair traits is a promising resource to improve P acquisition through breeding.

5.1.3. Reducing the metabolic costs of soil exploration

The production and maintenance of roots is associated with significant carbon costs. Consequently, aerenchyma formation may represent a beneficial adaptation to suboptimal availability of water or nutrients (Lynch, 1998; Fan *et al*., 2003; Lynch & Brown, 2008). There is considerable evidence for the induction of root cortical aerenchyma (RCA) when plants face unfavorable condition like low N, P, S and water availability, or high temperatures (Drew *et al*., 1989; Przywara & Stêpniewski, 2000; Bouranis *et al.,* 2003; Evans, 2003; Zhu *et al*., 2010). In soils with low P availability, RCA was correlated positively with maize root growth (Lynch, 2007). Saengwilai *et al*. (2014) found good correlations between RCA and rooting depth, N uptake, photosynthesis rate, biomass accumulation, and yield in maize grown in N-stress conditions. The probable explanation for these advantages is the reduction of metabolic costs for root formation and maintenance (Lynch, 2015).

5.1.4. Root exudates

A common mechanism of plants to cope with P limited environments is by root exudation to enhance availability of P. Root exudates include low molecular weight (*e.g*., organic acids, carboxylates, phosphatases, phenolic, and phytosiderophores) and high molecular weight compounds (*e.g*., mucilage, ectoenzymes) which are secreted into the soil by plant roots. These compounds account for 5 – 21% of the photosynthetically fixed carbon (Marschner, 1995). Root exudates play an important role in P fixing soils because

they can alter the bioavailability of P in the soil solution by solubilizing bound P in soil (Lynch, 2007). The type of exudate compounds and mechanisms plants use to solubilize P depends on plant species, plant nutritional status, and ambient soil conditions (Hinsinger, 2001). Among organic acids, citric acid, malic acid, and oxalic acid are the most important and commonly found in the rhizosphere (Jones, 1998). Citrate can free P from Al-P or Fe-P bound in acid soils and from Ca-P in calcareous soils or from rock phosphorus (Richardson *et al*., 2009b). Lupine species form cluster roots which can secrete citrate and malate (often called P solubilizers) in a sufficient amount which lowers the rhizosphere pH and enhances the availability of P for plant uptake (Braum and Helmke, 1995; Hocking and Jeffery, 2004).

Phosphorus starvation induced efflux of organic anions has been observed in several crops such as alfalfa (*Medicago sativa*), spinach (*Spinacia oleracea*), and radish (*Raphanus raphanistrum*) (Lipton *et al.*, 1987; Zhang *et al*., 1997; Gerke, 2015). White lupin (*Lupinus albus*) and common bean showed significant amounts of citric acids in the rhizosphere in response to P deficiency (Shen *et al*., 2002; Vance *et al*., 2003; Richardson *et al*., 2009a). Similarly, Hoffland *et al*. (2006) also recorded an 81% increase of organic acids exudation in lowland rice genotypes under P deficiency. Organic compounds secreted by plant roots into the rhizosphere can enhance the availability of fixed nutrients such as P for plants. Among different factors, such as plant species, plant age, temperature, light, soil moisture, and root damage, *etc*., microorganism's status in the rhizosphere is one that determines the kind and amount of organic compound secreted by plant roots (Rovira, 1969). However, while abundant evidence for enhanced root exudation in response to P deficiency exists, the

relationship between root exudation and P uptake may not be a universal relationship. Duputel *et al*. (2013) did not see an improvement in P availability in certain soil types when citrate efflux is increased, and Ryan *et al*. (2014) did not find a relationship between citrate efflux and P uptake in wheat.

Root secretion of acid phosphatase was examined as an adaptive mechanism of plants in response to P deficiencies in several crops (Todano and Sakai, 1991). For instance, under low P conditions, it was reported that large amounts acid phosphatases were secreted by rice roots (Hirata *et al*., 1982). In addition, studies on tomato and maize have confirmed the relationship between acid phosphatase activity and P-stress (Goldstein *et al*., 1988; Sachay *et al*., 1991).

5.1.5. Arbuscular mycorrhizal symbiosis

Arbuscular mycorrhizal fungi (AMF) belong to the phylum *Glomeromycota* (Schüßler *et al*., 2001). There are 323 species of AMF that have been described, (http://www.amf-phylogeny.com/amphylo_species.html, accessed 3rd December 2019). *Glomeromycetes* include *Glomus aggregatum*, *Glomus irregulare*, *Glomus rosea*, *Glomus mosseae*, and *Glomus etunicatum* (Giovannini *et al*., 2020). Species of *Glomeromycetes* differ in their spore size, color, and DNA structures. When spores germinate, the hyphae create a net called mycelium. The mycelium grows and contacts and penetrates the roots and grows in the root cortex and makes highly branched structures called arbuscules in the root cells. Arbuscules are covered by the membrane of the plant cell and exchange nutrients with the plants through transporters which are located on the plant cell membranes. Along with arbuscules, the AMF also produce vesicles which have a storage function.

Arbuscular mycorrhizal fungi play an important role in improving plant nutrient acquisition, particularly the acquisition of immobile nutrients like P (Cardoso and Kuyper, 2006; Smith and Read, 2008). The formation of a symbiotic relationship of plants with AMF is considered one strategy of plants to cope with nutrient deficiency, including for P, Zn, and Fe (Karandashov and Bucher, 2005; Smith and Read, 2008). Roots of more than 80% of terrestrial plant species form symbiotic relationships with AMF (Smith and Read, 2008). Arbuscular mycorrhizal fungi effectively extend the volume of soil that is explored for nutrients as they can enter small pore spaces and enhance access to limiting soil resources. The hyphae of mycorrhizal fungi are about 2 µm in diameter, about 10 times smaller than root hair (10 – 20 µm) and about 100 times smaller compared to fine roots (100 – 500 µm).

Once plants establish the relationship with AMF, P can be taken up by the roots directly or through the hyphae which extend the P depletion zone surrounding the roots (Smith *et al*., 2011). As part of the symbiotic relationship, AMF exchange uptake of water and nutrients for plant photosynthates (about 5 to 30% of total photosynthesis production) (Smith *et al.*, 2003). In addition to extending the P depletion zone, AMF hyphae also increase phosphatase activity, hydrolyze organic P and transfer the released P to plants (Joner and Johansen, 2000; Koide and Kabir, 2000). Evidence further indicates that AMF are not only improving plant growth, but also supporting diseases resistance in plants (Vigo *et al*., 2000; De la Pena *et al*., 2006).

5.2. Mechanisms to increase P utilization

5.2.1. Remobilization and internal use of P

Phosphorus is a constituent of many molecules including energy compounds adenosine mono-, di-, triphosphate (AMP, ADP, ATP) and reducing power (NADPH), (ii) nucleic acids (DNA & RNA), and (iii) phospholipids in cell membranes. Thus, it is essential for many processes including energy transmission/transfer, membrane and nucleotide synthesis, photosynthesis, and signal transduction (Plaxton and Tran, 2011; Havlin *et al*., 2014; Plaxton and Lambers, 2015).

Plants take up P from soil through the root system and then transport it to shoots and other organs in the xylem and phloem (Bowling, 1981; Clarkson, 1993). In this case roots act as a source for P that is delivered to shoots and other organs which are sinks for P and from which P may be re-translocated back to roots. Phosphorus availability plays a major role in the movement and allocation of P within plant (P influx and/or efflux) (Hamburger *et al*., 2002). In P starvation conditions, when P absorption is insufficient to satisfy the requirements for growth, P is remobilized from the pool previously accumulated in existing tissues to developing organs. Under P starvation, plants will mobilize and reuse the stored P in the vacuoles, plastids, and membranes (Schachtman *et al*., 1998).

Recently, it was found that microRNA isomers (including miRNA399 and miRNA827) and PHO2 are regulators of P uptake, sensing, and transport in plant cell, and are involved in P remobilization within the plant. They are components of the root to shoot P deficiency signaling pathway and help in maintaining P homeostasis in plants. Under P deficiency, they are upregulated and inhibit gene expression related

to P remobilization and responsiveness. miRNA399 targets the PHO2 gene encoding E2 enzyme that negatively regulates P uptake and root to shoot allocation (its suppression by miRNA399 will activate P uptake and root to shoot allocation) (Huang *et al*., 2013), while miRNA827 interacts with SPX-MSF genes which are relate to P sensing and transport (Hackenberg *et al*., 2013). In response to P starvation, miRNAs of the family 399 are able to bind and cleave the PHO2 transcript (Allen *et al*., 2005) or to cause translational repression of PHO2 (Bari *et al*., 2006; Chiou *et al*., 2006; Pant *et al*., 2009).

The repression of PHO2 expression by miRNA399 causes an increase in the expression of root Pi-uptake transporters (*e.g.* PHT1;8 and PHT1;9), and hence in the acquisition of Pi by the roots and its translocation to the shoot. In contrast to PHO1, which encodes an integral membrane protein and is involved in loading P into the xylem (Hamburger *et al*., 2002), PHO2 which encodes a ubiquitin-conjugating E2 enzyme (UBC24) has been implicated in protein degradation, (Aung *et al*., 2006; *Bari et al*., 2006). Indeed, PHO2 transcript levels increased remarkably in senescing leaves and maturating seeds (Bari *et al*., 2006).

Redistribution of P to growing tissues or replacement of phospholipids are considered as mechanisms of plant adaption to P starvation conditions (Adem *et al*., 2020). Plants expresses their tolerance to limited soil P availability by remobilization of P from senescent to growing tissues (Versaw and Harrison, 2002; Huang *et al*., 2011). Phosphorus, while relatively immobile in soil, is mobile in the plant, and is translocated from old to young leaves, which is the reason for the appearance of the first symptoms of P deficiency in older leaves (Dixon *et al*., 2020).

Low tissue P concentrations can be achieved by replacement of phospholipid by sulfolipids and galactolipids. Reduction in phospholipids and increases in sulfolipids and galactolipids in cell membranes, thylakoids or inner envelop membranes have been observed in several crops including barley and oats (*Avena sativa*) (Anderson *et al*., 2003; Tjellstro¨m *et al*., 2008), common bean roots (Russo *et al*., 2007), soybean (Gaude *et al*., 2004), and recently in proteaceases species (Lambers *et al*., 2012). Most species of Proteaceae are non-mycorrhizal plants (Brundrett, 2002) and very tolerant to impoverished soils not only because their cluster roots can release carboxylates to mine P from soil but also because of their ability to remobilize P within plants (Lambers *et al*., 2018).

5.2.2. Root: Shoot ratio

Depending on the availability of nutrients in the soil, plants allocate photosynthates to root or shoot tissues. In nutrient sufficient compared to nutrient limited conditions, a relatively higher proportion of photosynthates will be partitioning to the shoot rather than the root (Tilman, 1985). Indeed, increasing root to shoot dry weight is a common plant response to P deficiency (Hermans *et al*., 2006). Under P deficiency, an increase in root: shoot dry weight ratio was recorded in maize (Mollier and Pellerin, 1999), common bean (Nielsen *et al*., 2001), and soybean (Furlani *et al*., 2002). The allocation of carbohydrates to roots under P deficient conditions allows for enhanced root growth and consequently scavenging of more P from soil.

The relative growth of root and shoot are associated with PUE. To cope with limiting P soil, a larger proportion of carbohydrates is translocated to develop root

systems to improve the exploration of soil for P or improve P acquisition. This often goes along with increases in total root length, root surface area (Zhu and Lynch, 2004; Lynch and Ho, 2005).

6. Phosphorus use efficiency

6.1. Definition and calculation

The term nutrient use efficiency is mentioned in many reviews and is understood as the ability of a plant to acquire and utilize nutrients efficiently (Gourley *et al*., 1993). There are many different definitions for plant P use efficiency (PUE), but generally both P uptake/acquisition efficiency (PAE) and PUE contribute to total P efficiency (Vance *et al*., 2003; Rengel and Marschner, 2005). An efficient genotype can maintain high yield under low P conditions. Acquisition efficiency is the ability of plant roots to uptake P from soil, while P utilization efficiency is generally defined as the ability of a plant to efficiently use the P taken up in terms of dry biomass or yield production per amount of P in the plant (Asher and Loneragan, 1967; Aziz *et al*., 2014).

That said, there are a number of definitions/calculations for PUE that can lead to some confusion (McLachlan, 1976; Siddiqi and Glass, 1981; Fohse *et al*., 1988; Godwin and Blair, 1991; Baligar *et al*., 2001; Fageria *et al*., 2013). A survey of terminology and associated definitions and the relevant citations are listed in Table 1-1. In this study, PUE is understood as the amount of biomass (g) produced per unit P contained in the plant (g plant^{-1}), where, in most experiments, only shoot biomass and shoot P content were determined. As such, PUE in the data chapters generally refers to the amount of shoot biomass produced per shoot P content. Ideally, we would

have liked to express PUE as the ratio of total plant biomass and total P uptake, but, both root biomass as well as the amount of P taken up by the plant and contained in root tissue is difficult to quantify accurately.

Table 1-1. Common definitions and calculations of P efficiency terms and associated references.

Term	Definition	Calculation/Formula	References
P efficiency ratio	The amount of plant dry matter per unit of P uptake	Dry matter (g)/ g P uptake in plant	Fohse *et al*., 1988
P utilization efficiency	Shoot dry weight per unit of P uptake	Shoot biomass (g)/ shoot P content (mg)	Osborne and Rengel, 2002
P uptake efficiencies	Accumulation of P per unit of root weight	mg P / g fine root dry matter	Blair and Cordero, 1978; Elliott and Lauchli, 1985
Physiological P use efficiency	Plant yield production per unit of P uptake	Dry weight of grain or shoot in kg/ P uptake in grain or shoot in kg	Baligar *et al*., 2001; Fageria *et al*., 2013
Agronomic P efficiency	Yield increases per unit of P present in soil	(Yield in high P conditions – Yield in low P condition)/ P available in soil	White and Hammond, 2008

6.2. Genetic variation for phosphorus use efficiency

Genetic variation for P efficiency, which the criterion used to discriminate genotypes, was dry weight production per unit of available P in common bean has been demonstrated over 25 years ago (Whiteaker *et al*., 1976). Subsequent studies showed that such variation was heritable and was related to root traits. Lynch and co-workers have shown substantial variation in common bean P efficiency, and it was found to be stable across soil environments in Latin America where they express this as growth and yield in relation to available P from soil pools or soil amendments, which incorporates the ability to yield at low P with responsiveness to fertilizer inputs. They expressed P efficiency as growth and yield in relation to available P from soil pools or soil amendments, which incorporates the ability to yield at low P with responsiveness to fertilizer inputs (Lynch and Beebe, 1995).

Plants differ in their response to contrasting P supply (Aziz *et al*., 2014) and PUE related traits are genetically heritable (Nielsen and Schjorring, 1983; Jones *et al*., 1989). This variability can be useful for crop improvement through breeding programs to tackle the problem of P-deficient soils (Fageria and Baligar, 1993). Studies on pigeon pea (Ae *et al.,* 1990, 1993), lupin (*Lypinus albus* L.), and alfalfa (*Medicago sativa* L.) (Gardner *et al*., 1982) indicate that these species have the ability to absorb and free P from the Fe-P bound form in an Alfisol soil. The explanation for the superior acquisition of P from low P soils is the ability to secrete some organic acids such as piscidic acid and its derivatives which can release P from Fe-P by chelating with Fe^{3+}, or by association with AMF that will extend the P absorptive area (Ae *et al*., 1993).

Large genotypic variation in PUE related traits has been observed in many crops (Gerloff, 1976; Gerloff and Gabelman, 1983; Alam, 2003; Kosar *et al*., 2003; Hidaka and Kitayama, 2009; Sulpice *et al*., 2014; Keneni *et al*., 2015; and Iqbal *et al*., 2019), including for soybean (Pan *et al*., 2008; Zhou *et al*., 2016). Understanding the genetic variability of PUE related traits is the key to determine mechanisms of plant adaption to low P soil conditions, and in turn incorporation of desired traits into elite germplasm improve yield in P limited environments.

6.3. Quantitative trait loci in breeding of PUE plants

The concepts for detecting a quantitative trait locus (QTL) were first noticed by Geldermann (1975). It is defined as a region of the genome that is associated with an effect on a quantitative trait. Quantitative trait locus analysis is a means to identify links between genotypes and phenotypes of a quantitative trait in segregating populations by applying appropriate statistical methods.

Phosphorus efficiency-related traits are inherited as quantitative traits controlled by multiple genes and highly affected by environments (James *et al*., 2016; Van de Wiel *et al*., 2016). Genetic mapping studies of various traits related to low P-stress tolerance have been carried out in several important agricultural crops including rice, maize, soybean, common bean, rape seed, wheat, barley, and pearl millet (Maharajan *et al*., 2018).

To date, in soybean, a number of QTLs identified for traits used for selection of P-efficient germplasm (Maharajan *et al*., 2018). For examples, QTLs were reported for root hair length and density (Wang *et al*., 2004); or eleven traits including days to flowering, day to maturity, plant height, number of nodes on main stem, protein and

oil content, pods per node, lodging, 100-seed weight, and yield (Zhang *et al*., 2004). Study of Li *et al*. (2005) detected seven QTLs and mapped on two linkage groups for three traits of shoot fresh weight, root P content, leaf P content. Besides, thirty-four additive QTLs were found on nine linkage groups for shoot dry weight, root dry weight, total plant dry weight, PUE, PAE of soybean at seedling stage (Zhang *et al*., 2009). In their other report, thirteen QTLs associated with flower and pod abscission rate were recorded (Zhang *et al*., 2010). Moreover, QTLs for shoot, root, pod dry weight, root to shoot ratio, root width, root surface area, shoot and root P content and yield (seed number and seed weight) (Liang *et al*., 2010); plant height, total dry weight, PAE, PUE, phosphorus concentration and acid phosphatase activity (Zhang *et al*., 2016); and phosphorus concentration, phosphorus uptake, phosphorus utilization efficiency, and other photosynthesis-related traits (Li *et al*., 2016) were also identified.

Obviously, soybean is an important legume plant and identification of QTLs provides useful information for marker-assisted selection which can accelerate the development of more P-efficient germplasm.

7. Rationale for Research

Although many studies on PUE in plants exist, including a number of them for soybean, characterization of genetic variation in P uptake as well as P use efficiency in soybean is needed to identify suitable genotypes for breeding of soybean cultivars with superior 1) ability to acquire P, particularly in soils with low P availability, and 2) production of biomass and yield per unit of P taken up. Additionally, the identification of soybean genotypes contrasting in P uptake and utilization efficiencies will set the stage for

comparative studies into the physiological, biochemical and molecular mechanisms underpinning these P-related phenotypes and will allow the development of resources such as biparental mapping populations or multiparent advanced generation intercross (MAGIC) populations to explore the genetic underpinnings of P uptake and P use efficiencies. Thus, the research described in the following chapters provides valuable information required for breeding of more P efficient soybean genotypes, the most eco-efficient way to overcome the looming P availability crisis. Ultimately, soybean cultivars bred for superior P acquisition and utilization efficiencies are expected to limit the overuse of P fertilizer in soybean production, increase economic returns for farmers, and enhance environmental sustainability of soybean production.

8. Objectives

The purpose of this study was to evaluate P uptake and PUE of diverse soybean genotypes and to explore relationships between root system characteristics and of P acquisition and PUE in soybean. Specifically, the objectives were to:

1) Identify soybean genotypes with contrasting in P acquisition and in P use efficiency.
2) Evaluate identified soybean genotypes for their responses to differences in P availability.
3) Characterize top-soil root system characteristics and arbuscular mycorrhizal fungi infection of selected contrasting soybean genotypes.

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CHAPTER 2

CHARACTERIZATION OF SHOOT P CONCENTRATION AND ITS RELATIONSHIP WITH TOPSOIL ROOT ARCHITECHTURES IN SOYBEAN

ABSTRACT

Phosphorus (P) plays a vital role in a plant's growth and development. The objectives of this research were to evaluate soybean genotypes for biomass production, shoot P concentration, and P accumulation in relation with top-soil root characteristics. Forty-one nested association mapping (NAM) parents were grown under field conditions in two years 2014, 2015, and in 2016, 20 selected genotypes were investigated for top-soil root architecture. At the mid pod fill stage, shoot biomass was sampled and analyzed for P concentration and content. In 2016, roots of plants at the mid pod fill stage were excavated and stem diameter, tap root diameter, nodule size, nodule density, number of adventitious/ upper roots, lateral root density on upper lateral roots, number of lower roots, lateral root density on lower lateral roots, angle of lateral roots, and overall complexity of the root system were assessed. Significant genetic variability was observed for shoot biomass, shoot P concentration, and shoot P content among the 41 NAM parents. Selected genotypes differed significantly in the different root traits except for lateral root density on lower lateral roots, angle of upper lateral roots, number of upper lateral roots, and tap root diameter. Shoot biomass was positively related with shoot P content and overall complexity of the root system but shoot P concentration was negatively correlated with shoot PUE. Genotypes contrasting in P-related traits as well as root system traits are useful for further studies of P use efficiency mechanisms and for germplasm improvement efforts.

INTRODUCTION

Among different macronutrients, phosphorus (P) plays a vital role in plant growth and development. Phosphorus is an essential component of molecules such as nucleic acids, phospholipids, and ATP. Unlike N, P is only available from soil and is taken up by plants in the orthophosphate form through the root systems. Phosphorus is abundant in soil, but it is mostly in insoluble forms, such that fertilizer applications are often necessary to satisfy plant needs. However, the use of P fertilizers raises some concerns, namely, the mining of rock phosphate is neither eco-friendly nor sustainable in the long term. It is estimated that the rock phosphate reserves will be depleted in about 500 – 600 years (Sharma *et al.*, 2013). The world phosphate rock reserves are mainly distributed in China, Morocco, and the United States of America. It is very rare in many areas of the world, such as Europe or West, Central Africa.

Soybean (*Glycine max* L. Merrill) is one of the most valuable crops in the world and could possibly become a major crop in Africa due to its many uses as a source of protein and for livestock, aquaculture and for the human diet. However, the production of soybean can be severely limited by low P availability. To overcome this constraint, plants express several adaptations to enhance P uptake and P internal utilization efficiency. Researchers showed that grain yield has a good relationship with above grown biomass and plant growth stages are well coordinated with high yield and high P efficiency (Fageria *et al*., 2013; Sandana and Pinochet, 2014). Genotypic variation for soybean P uptake and P use efficiency have been documented previously (Pan *et al*., 2008; Zhou *et al.*, 2016). Ao *et al*. (2014) observed a significant difference of the accumulation in dry matter and

harvest index of high P efficiency soybean genotypes compared to low P efficiency genotypes.

Plant roots play a vital role in water and nutrient uptake. Root architecture is the spatial distribution of roots in the soil profile. Root system architecture is affected both by genetics and environment. In response to P deficient conditions, plants exhibit varied responses including alterations in root morphology (Wissuwa, 2003), exudation of organic acids into the rhizosphere to solubilize mineral and organic P sources (Johnson *et al*., 1996), exudation of phosphatases to mineralize organic P in soil (Li *et al*., 2012). Thus, understanding root system characteristics in general and in response to nutrient limitations in particular is of great importance to breed cultivars that perform better under nutrient limited conditions. However, the evaluation of root traits, especially under field conditions, is difficult and labor intensive. Consequently, there is comparatively limited work on root characteristics that has been conducted for US soybean germplasm to explore the relationship between root and shoot traits in general and P uptake and accumulation specifically. Considering the importance of root architecture, and the accumulation of P in the topsoil layer, a focus on topsoil root architecture is warranted, particularly since the top-soil root system characteristics are known to influence P acquisition in Chinese soybean germplasm (Zhao *et al*., 2004) as well as in common bean (Lynch and Beebe, 1995; Bonser *et al*., 1996; Liao *et al*., 2001; and Lynch and Brown, 2001).

Our aim was to characterize shoot P concentration, shoot P content, and shoot based P use efficiency of the soybean nested association mapping (SoyNAM) parental lines (Experiment 1), and then select a subset of contrasting SoyNAM parental lines and additional plant introductions to examine the relationships of shoot P traits with topsoil

root architecture characteristics under field conditions (Experiment 2). We hypothesized that soybean genotypes contrasting for shoot P phenotypes show consistent differences in one or more root traits.

MATERIALS AND METHODS

Field experiments were carried out to characterize P status in soybean shoot tissues and the relationship with topsoil root architecture. The experiments were carried out at Rollins Bottom (38°55'41.8"N 92°21'09.8"W) on a Haymond silt loam soil (coarse-silty, mixed super active mesic Dystric Fluventic Eutrudepts), in Columbia, MO.

Experiment 1: Forty-one SoyNAM parental lines (Diers *et al*., 2018) were planted on 7 May 2014 and 28 May 2015 in 3.05-m long single row plots with a spacing of 0.76-m between rows. Seeds were planted approximately 2.5 cm deep at a density of 34.5 seeds per m^2.

Experiment 2: Twenty soybean genotypes selected based on the SoyNAM experiments in 2014 and 2015 and other preliminary data were planted on 1 June 2016 as described for Study 1. Genotypes were selected based on shoot P concentration, biomass production, and root complexity, and included six NAM parents, 3 identified to have low shoot P concentration (S06-13640; LG05-4317; LG05-4464) and 3 with high shoot P concentration (CLOJ095-4-6; PI561370; 4J105-3-4), the SoyNAM hub parent (IA3023), eight plant introductions (PI) lines [4 low shoot P concentration (PI603454; PI399027; PI423890C; PI417107) and 4 high shoot P concentration (PI424614; PI603171; PI408255B; PI398965)], two obsolete public varieties which can be transformed (Maverick and Magellan), and three genotypes obtained from the USDA because they were identified to perform well under low-P conditions in Africa (H7, H10, and H17). Four of

the eight PIs included (PI603454; PI399027; PI424614; PI603171) are parents of mapping populations under development.

All experiments were laid out in a randomized complete block design with four replications. No pesticide applications were conducted, and weeds were controlled by hoeing as needed. No fertilizer applications were conducted as soil tests results did not indicate a need for application based on University of Missouri Extension recommendations (Table 2-1).

Data collection:

At the mid seed filling (R5.5) (Fehr and Caviness, 1977) growth stage, five representative plants in each plot were cut two to three centimeters above the soil surface and dried at 65°C to a constant weight. Samples were weighed (except in 2014) and ground to a fine powder. Initially, samples were ground through a 2 mm screen using a Wiley mill (Thomas Sientific, Swedesboro, NJ, USA). The resulting ground material was thoroughly mixed, and a subsample was processed with a Cylone mill (Cyclotech, Foss North America) using a 1 mm screen. The ground samples were sent to analysis for P concentrations using a Spectro ARCOS ICP-OES at the Agriculture Diagnostics Lab of the University of Arkansas, Fayetteville, AR.

Shoot P content was calculated by multiplying shoot P concentration with shoot dry weight. Physiological P use efficiency was defined as the mg of shoot dry weight produced per mg of P absorbed by plants. P content in the shoot (mg plant^{-1}) was measured and used as a surrogate for P uptake because plant may shed leaves that contain P that was taken up by the plant and accurate quantification of root biomass is very difficult under field conditions.

In Experiment 2, right after biomass sampling at R5.5, five different representative plants in the middle of each plot were selected and the root system were extracted from the soil. To this end, a soil column of 20 cm x 20 cm x 30 cm with the plant base at the center was dug to recover roots in the top portion of the soil profile. The soil was manually removed from the roots with great care. In turn, eleven characteristics including stem diameter, tap root diameter, nodule size, nodule density, number of upper lateral roots, lateral root density of upper lateral roots, number of lower lateral roots, lateral root density of lower lateral roots, angle of upper lateral roots, angle of lower lateral roots, and root overall complexity were assessed as described in Table 2-2. The remaining plants were harvested for seed increasing at maturity (R8) for use in future experiments.

Statistical analysis:

One-way analysis of variance (One-way ANOVA) was conducted using the PROC GLM model in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), where genotype was the fixed effect and replication was the random effect. Mean separation was conducted using Fisher's protected by least significant difference (LSD) at the $P < 0.05$ level.

Principal component analysis was applied by PROC PRINQUAL to investigate the possibility of clustering of genotypes and root traits in Experiment 2.

RESULTS

Experiment 1: Characterization of shoot P traits in SoyNAM parental lines

Soybean genotypes differed significantly for shoot P concentration in 2014 and 2015, and for shoot biomass, shoot P content, and shoot PUE ($p < 0.05$) in 2015 (Table 2-3). Although significant genotype and year effects were observed, significant genotype by

year interaction was observed ($p < 0.05$) for shoot P concentration which ranged from 1.86 g kg^{-1} to 2.58 g kg^{-1} in 2014 and from 2.29 g kg^{-1} to 3.36 g kg^{-1} in 2015 experiment. The two genotypes with the greatest average shoot P concentration for the two years were PI5613370 (2.89 g kg^{-1}) and 4J05-3-4 (2.93 g kg^{-1}), and the two genotypes with the lowest average P concentrations were S06-13640 (2.19 g kg^{-1}) and LG05-4317 (2.25 g kg^{-1}).

Due to lack of data on shoot biomass in 2014, shoot biomass, shoot P content, and PUE only are available for 2015. Shoot dry weights ranged from 27.60 to 53.65 g $plant^{-1}$. The highest shoot biomass was recorded for genotype LG054317 (53.65 g $plant^{-1}$), followed by Maverick, and CLOJ095-4-6 with 51.3, 48.8, g $plant^{-1}$, respectively. The genotypes with the lowest shoot biomass accumulation were PI581751 (27.6 g $plant^{-1}$), PI507681B (32.05 g $plant^{-1}$), Skylla (33.75 g $plant^{-1}$), and LG90-2250 (32.90 g $plant^{-1}$) (Table 2-3).

Shoot P content ranged from 0.079 to 0.164 g $plant^{-1}$ with 4J05-3-4 and CLOJ095-4-6 having the highest (0.164, 0.158 g $plant^{-1}$, respectively) and PI518751 and S06-13640 the lowest shoot P contents (0.079, 0.08 g $plant^{-1}$, respectively). In contrast, PUE which ranged from 298.66 to 441.85 g BM g P^{-1}, was lowest for 4J05-3-4 and CLOJ095-4-6 (298.66 and 300.18 g BM g P^{-1}, respectively and highest for LG05-4317 and S06-13640 (441.85, 433.11 g BM g P^{-1}, respectively). The correlation between PUE and shoot P concentration was highly negative ($r = -0.9872$). Interestingly, shoot biomass was not significant correlated with shoot PUE and shoot P concentration in this experiment, but it was significantly positively associated with shoot P content ($r = 0.90715$) (Table 2-4).

Experiment 2: Characterization of shoot P traits, root traits, and their relationships in soybean genotypes selected for differences in shoot P traits

Analysis of variance indicated significant genotypic differences for shoot P concentration and shoot P use efficiency but not for shoot biomass production and shoot P content (Table 2-5; Table 2-6). In terms of root traits, genotypes differed only for Overall complexity, Nodule size, Nodule density, Number of lower lateral roots, and Stem diameter immediately above the root stem intersection (Table 2-5).

Plant growth and nutrient accumulation:

Shoot P concentrations among the 20 genotypes ranged from 1.93 g kg^{-1} to 3.25 g kg^{-1} with PI398965 and CLOJ095-4-6 containing significantly higher concentrations than the other genotypes while H10 had the lowest shoot P concentration followed by H17 (Table 2-6). The low shoot P concentration likely reflects a dilution effect as H10 had the greatest shoot biomass (95.85 g $plant^{-1}$). With 56.950 g $plant^{-1}$, PI424614 produced the lowest shoot BM and also had to lowest shoot P content (0.159 g $plant^{-1}$). PI417107 accumulated the largest amount of P in the shoot tissue (0.243 g $plant^{-1}$).

PUE reflects how well cultivars use P accumulated in shoot tissue with respect to shoot biomass production. PUE values obtained in Experiment 2 were similar to those observed in Experiment 1 and ranged from 311 g BM g P^{-1} to 527 g BM g P^{-1}. Consistent with strong negative correlations between shoot P concentration and PUE, the two genotypes H10 and H17 which exhibited the lowest shoot P concentrations (1.93 g kg^{-1} and 2. 38 g kg^{-1},

respectively), had the highest PUE (527 g BM g P^{-1} and 434 g BM g P^{-1}, respectively) (Table 2-6).

Examination of the top-soil root system characteristics of the 20 entries revealed high coefficients of variation (CV) for all root traits (Table 2-7). This was not surprising given the plasticity of root growth and the challenges associated with phenotyping of root systems of field-grown plants. Nonetheless, significant genotypic variation for Overall complexity, Nodule size, Nodule density, Number of lower lateral roots, and Stem diameter (Table 2-5). The genotype effect for Overall root complexity, a score that attempts to reflect root length density in the topsoil, was the most significant among all traits ($P < 0.0001$). A wide relative range (>35%) was observed for all root traits and at a threshold of $P < 0.10$, the Angle of lower lateral roots and Lateral root density on upper lateral roots also were significant (Tables 2-5; Table 2-7; Table 2-8).

Relations among root and shoot traits:

The relationships among the 11 top-soil root system traits and between them and shoot BM, shoot P concentration, shoot P content, and shoot PUE were examined by correlation analysis (Table 2-8) and principal component analysis (Figure 2-1). Among all relationships, those of particular interest are the ones between root system traits and shoot P concentration, shoot P content, and shoot PUE. However, only a limited number of significant correlations were found, namely a positive correlation between Overall complexity of the root system with shoot P content (r = 0.214) and Lower lateral root angle with shoot P concentration (r = 0.311), Shoot P content (r = 0.244), and shoot PUE (r = -0.240). Consistent with results from Experiment 1, the relationships of shoot biomass with

shoot P content (r = 0.674), and PUE with shoot P concentration (r = -0.968), and shoot P content (r = -0.597) were strong.

To further evaluate relationships among traits of all 20 genotypes, Principal Component Analysis (PCA) was conducted and a biplot was generated (Figure 2-1). This analysis revealed that the first two principal components explained 67.25% of the observed variance (Figure 2-1). The first component (PC1) represented 37.82% of the variability and accounted primarily for shoot BM, shoot P concentration, shoot PUE, Lateral root density on upper lateral roots, Angle of upper lateral roots, Angle of lower lateral roots, and Number of lower lateral roots. The second principal component (PC2) represented 29.43% of the variability, largely defined by Overall complexity, Stem and Tap root diameters, Lateral root density on lower lateral roots, and nodule size and density (Figure 2-1).

DISCUSSION

Significant genotypic variation in shoot P traits were observed in both experiments. Additionally, genotypic variation in root system architecture was documented in Experiment 2. For the genotypes included in Experiment 1 and Experiment 2, Maverick, CLOJ095-4-6 and 4J105-3-4 consistently ranked high for shoot BM, shoot P concentration, shoot P content, while S06-13640, LG05-4317 had higher shoot PUE in both experiments. As such, these results are consistent with considerable genotypic control over shoot P content, shoot P concentration and shoot PUE. Shoot P concentrations and shoot PUE observed in this study were similar to those reported by others for pot experiments (Shujie and Yunfa, 2011; Vandamme *et al*., 2013). They found that shoot P concentration ranged from 1.15 to 4.40 mg g^{-1} whereas shoot PUE ranged from 0.3 to 0.6 g BM mg P^{-1}, respectively.

Root system architecture and morphology differ dramatically among species and within species (Chloupek *et al*., 2006; Manschadi 2008; Hammond *et al*., 2009; Hargreaves *et al*., 2009), and plays an important role in nutrients and water uptake (Lynch, 1995). Several studies have shown good correlation between root architecture and nutrient uptake, with root length, root surface area, number of lateral roots, representing particularly important traits (Manschadi, 2008; Hargreaves *et al*., 2009). For soybean grown under field conditions in a typical acidic red soil, Ao *et al.* (2010) studied on 88 recombinant inbred lines (RILs) of F11 generations, derived from two parent CN4 and XN6, and found significant positive correlations between root length, root depth, root surface area, and root width with P content, suggesting the important of root morph-architecture traits for improving P efficiency in this crop. For common bean, Bonser *et al*. (1996) reported that plants with shallower root systems were better adapted to conditions of low soil P availability as P is more prevalent in the topsoil layers.

In this study, overall complexity of the root system was positively correlated with shoot P content ($r = 0.214$) and the angle of the lower lateral roots was positively correlated with shoot P concentration ($r = 0.311$) and shoot P content ($r = 0.244$) and negatively correlated with shoot PUE ($r = -0.240$) (Table 2-8). Although not very high, these correlations are consistent with an important influence of root system architecture on soybean P uptake.

It is important to note that all experiments were conducted under conditions of sufficient nutrient availability as indicated by soil test results. As such, the genetic variation observed for all examined traits indicates opportunities for genetic improvement of soybean P use efficiency under fertility conditions currently targeted by US soybean

farmers. Additional experiments are needed to explore whether and to what extent the genotypes studied here respond under conditions of low P availability, and whether relationships of root system architecture traits with P uptake and PUE change as a function of soil P conditions.

This study identified soybean genotypes that 1) consistently differ in shoot P-related traits, and 2) exhibit large variation in root system architecture under P-sufficient conditions. Genotypes examined in this study that are contrasting for different traits will be valuable for future studies aimed at dissecting the mechanism associated with PUE. Further, the results presented here set the stage for breeding of cultivars with improved P uptake and PUE traits as well as the dissection of the genetic mechanisms underpinning these traits in soybean, which will be accelerated since this study included SoyNAM parental lines as well as parents of biparental mapping populations that already are under development.

Overall root complexity, a score that attempts to reflect root length density in the topsoil, also called “fibrous roots”, we observed a great variation in root overall of soybean genotypes ($P < 0.0001$). However, we did not see a significant correlation between root overall and shoot biomass or shoot P accumulation.

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TABLES AND FIGURES

Table 2-1. Soil physical and chemical properties at the field site at Rollins Bottom in Columbia, Missouri, in 2015 and 2016.

Soil test information	2015	2016
pHs (salt pH)	6.6	6.4
Phosphorus (kg $ha^{-1)}$	90.79	100.88
Potassium (kg ha^{-1})	173.73	167.01
Calcium (kg ha^{-1})	2935.51	3565.43
Magnesium	229	217
Organic matter (%)	1.0	1.1
Neutralizable acidity (meq/100g)	0.0	0.0
Cation exchange capacity (meq/100g)	7.7	7.5

Table 2-2. Description of soybean root system traits and how they were assessed.

Name of traits (unit)	Abbreviated	Rating methodology or score
Stem diameter (mm)	StemDia	Diameter of the stem right above root/shoot zone in (mm)
Taproot diameter (mm)	TapRDia	Measured the diameter of tap root at five cm below the upper lateral roots
Root complexity score (1-5)	Overall	Visual rating of the overall complexity: 1 = very simple (least fibrous), 2 = simple, 3 = average, 4 = complex, 5 = very complex (most fibrous).
Nodule size (mm)	NodSize	Visual estimate based on a scale consisting of four reference dots with diameters of 0.5, 1, 1.5, 2 (mm). Estimate based on 5 nodules representing the visual majority of the nodules.
Nodule density score (1-5)	NodDen	Visual rating based on a scale of 0 to 5 (no nodules, very few nodules, few nodules, medium number of nodules, many nodules, and very dense nodulation, respectively)
Number of lateral upper roots (No.)	NADUpRoot	First order lateral roots were counted in to the 20 cm of dug root
Lateral root density (No.) Adv/Up	LatRootDenAdUp	Number of the second order lateral roots within a 2 – 4 cm section of the biggest randomly picked lateral root with the section starting at a distance of 2 – 4 cm from the main taproot
Angle of lateral root upper (º)	AngAdUp	The angle of lateral roots relative to the soil level with 90° representing the angle of the taproot orientated vertically. Angle of 5 upper roots to the nearest 50 at 5 cm away from stem or tip if root is shorter than 5 cm, bigger diameter roots prioritized for measurements. Angles were determined using a plexiglass board made into a 180° protractor with angles indicated every 5°.
Number lower roots	NLowRoot	Number of lower roots
Lateral root density lower	LatRootDenLow	Number of lateral roots in the 2 – 4 cm zone from one randomly selected lower root
Angles of lower root (º)	AngLow	Angle of 5 lower roots to the nearest 50 at 5 cm away from stem or tip if root is shorter than 5 cm, bigger diameter roots prioritized for measurements, if there are fewer than 5 roots use (.) for any angles beyond number roots present.

Table 2-3. Shoot biomass, shoot P concentration, shoot P content, and shoot P use efficiency (PUE) of SoyNAM genotypes grown at Rollins Bottom in 2014 and 2015.

Genotype	Shoot P concentration (%)				Shoot biomass (g)		Shoot P content (g plant^{-1})		PUE (g BM g P^{-1})	
	2014		2015		2015		2015		2015	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
4J105-3-4	0.250	0.008	0.336	0.010	48.700	4.097	0.164	0.017	298.664	9.287
5M20-2-5-2	0.217	0.026	0.282	0.011	39.200	3.254	0.111	0.013	356.658	14.620
CL0J095-4-6	0.253	0.006	0.325	0.009	48.800	0.337	0.158	0.004	308.830	8.271
CL0J173-6-8	0.243	0.013	0.334	0.010	40.250	1.603	0.134	0.004	300.180	8.845
HS6-3976	0.198	0.010	0.311	0.006	39.200	1.131	0.122	0.004	322.374	5.750
IA3023	0.227	0.012	0.295	0.008	34.050	5.354	0.101	0.018	340.247	8.722
LD00-3309	0.224	0.012	0.271	0.010	37.800	1.149	0.103	0.007	371.184	13.156
LD01-5907	0.251	0.011	0.308	0.003	36.400	0.316	0.112	0.002	324.523	3.438
LD02-4485	0.235	0.011	0.311	0.026	41.350	3.399	0.128	0.013	328.390	25.058
LD02-9050	0.246	0.016	0.313	0.005	38.600	0.141	0.121	0.002	319.484	5.122
LG00-3372	0.207	0.011	0.264	0.003	42.850	1.452	0.113	0.005	378.937	4.333
LG03-2979	0.220	0.014	0.321	0.008	45.050	1.147	0.144	0.003	312.610	7.889
LG03-3191	0.207	0.008	0.278	0.004	34.000	0.909	0.094	0.002	360.217	4.644
LG04-4717	0.222	0.014	0.293	0.012	37.800	2.881	0.111	0.011	343.770	15.305
LG04-6000	0.226	0.009	0.308	0.006	41.500	1.313	0.128	0.006	324.735	5.976
LG05-4292	0.233	0.014	0.314	0.004	40.350	4.014	0.126	0.012	318.857	3.751
LG05-4317	0.222	0.015	0.229	0.013	53.650	7.962	0.125	0.025	441.847	24.397
LG05-4464	0.196	0.004	0.273	0.008	39.200	4.710	0.107	0.014	367.564	10.743
LG05-4832	0.206	0.014	0.307	0.011	45.450	7.722	0.142	0.028	327.616	12.495

LG90-2550	0.239	0.019	0.299	0.009	32.900	2.783	0.098	0.006	335.035	9.913
LG92-1255	0.235	0.011	0.294	0.006	37.850	3.725	0.112	0.013	340.774	6.189
LG94-1128	0.242	0.029	0.275	0.010	36.550	2.660	0.101	0.010	364.689	13.255
LG94-1906	0.213	0.009	0.254	0.006	40.550	4.054	0.103	0.011	393.889	8.594
LG97-7012	0.253	0.011	0.306	0.007	41.950	4.995	0.128	0.015	327.346	7.866
LG98-1605	0.214	0.009	0.301	0.012	31.800	2.005	0.096	0.007	334.325	12.727
Magellan	0.215	0.009	0.276	0.008	45.000	4.138	0.124	0.012	363.549	10.573
Maverick	0.221	0.011	0.286	0.015	51.300	7.684	0.147	0.025	353.252	18.716
NE3001	0.226	0.007	0.315	0.007	34.000	4.293	0.107	0.014	318.423	6.948
PI398881	0.219	0.011	0.286	0.006	44.050	2.956	0.126	0.007	349.892	8.121
PI404188A	0.204	0.015	0.267	0.006	40.050	6.270	0.107	0.016	375.397	7.905
PI427136	0.186	0.014	0.298	0.013	48.000	3.410	0.143	0.011	337.550	13.413
PI437169B	0.222	0.016	0.254	0.015	38.050	2.027	0.097	0.010	397.418	24.004
PI507681B	0.249	0.022	0.263	0.009	32.050	4.083	0.083	0.008	381.862	12.474
PI518751	0.215	0.014	0.291	0.012	27.600	5.233	0.079	0.014	345.168	14.696
PI561370	0.258	0.026	0.321	0.003	44.000	1.683	0.141	0.005	311.390	3.311
PI574486	0.238	0.015	0.278	0.009	37.050	1.994	0.104	0.008	360.564	11.996
Prohio	0.231	0.014	0.300	0.003	37.200	1.317	0.112	0.004	333.685	2.856
S06-13640	0.207	0.007	0.231	0.005	34.500	1.636	0.080	0.003	433.113	9.966
Skylla	0.226	0.016	0.293	0.012	33.750	6.385	0.100	0.021	342.718	14.543
TN05-3027	0.218	0.004	0.293	0.005	47.000	3.831	0.137	0.009	341.639	6.257
U03-100612	0.232	0.022	0.295	0.013	39.200	3.359	0.115	0.010	340.555	14.117
Mean	0.225		0.291		39.966		0.117		347.047	
LSD	0.038		0.026		10.730		0.035		33.495	
CV%	12.034		9.738		16.912		20.482		9.976	
P values										
Genotypes (G)	0.0273		<0.0001		0.0006		<0.0001		<0.0001	

Table 2-4. Correlation coefficients for relationships among shoot biomass, shoot P concentration, shoot P content, and shoot P use efficiency (PUE) in SoyNAM genotypes grown at Rollins Bottom in 2015.

	Shoot biomass (g)	Shoot P concentration (%)	Shoot P uptake (g plant^{-1})	PUE (g BM g P^{-1})
Shoot biomass (g)	1			
Shoot P concentration (%)	0.11686^{NS}			
Shoot P uptake (g plant^{-1})	**0.90715***	**0.51538***		
PUE (gBM gP^{-1})	-0.0999^{NS}	**-0.9872***	**-0.494***	1

Note: * Significant at *P = 0.05*

NS Non-significant

Table 2-5. Analysis of variance results for the effect of genotype for root and P traits for plants growth at Rollins Bottom in 2016.

No.	Sources	df	*P*-value	Coeff Var	Mean	LSD
1	StemDia	19	0.0228	13.3298	9.4709	1.8031
2	TapRDia	19	0.1084	17.1992	5.7481	1.4120
3	Overall	19	<0.0001	17.0140	2.5633	0.6229
4	NodSize	19	0.0005	15.0387	2.6646	0.5723
5	AngLow	19	0.0513	25.4714	20.1929	7.3461
6	NodDen	19	0.0021	23.0430	2.2667	0.7524
7	NADUpRoot	19	0.1896	52.0724	4.5065	3.4089
8	LatRootDenAdUp	19	0.0604	27.2463	7.1068	2.8664
9	AngAdUp	19	0.5020	78.1940	2.6987	3.2654
10	NLowRoot	19	0.0018	20.3293	17.2857	5.1451
11	LatRootDenLow	19	0.5404	17.2751	8.2187	2.0968
12	ShootBM	19	0.2966	17.7106	74.8885	19.2590
13	Pconc	19	0.0230	16.5134	0.2761	0.0646
14	Pcont	19	0.6274	25.1399	0.2089	0.0744
15	PUE	19	0.0052	16.9059	376.7275	90.1810

Table 2-6. Shoot biomass production, shoot P concentration, shoot P content, and shoot P use efficiency of 20 soybean genotypes grown at Rollins Bottom in 2016.

Gen	Shoot biomass (g)	Shoot P concentration (g kg^{-1})	Shoot P content (g $plant^{-1}$)	PUE (g BM gP^{-1})
4J105-3-4	74.35BCD	2.92ABC	0.218ABC	343CDE
CLOJ095-4-6	74.75BCD	3.24A	0.243A	311E
H10	95.85A	1.93D	0.219ABC	527A
H17	82.40ABC	2.38CD	0.199ABC	434B
H7	86.61AB	2.84ABC	0.241AB	364BCDE
IA3023	70.95BCD	2.83ABC	0.203ABC	358BCDE
LG05-4466	79.00ABC	2.47CD	0.197ABC	413AB
LG05-4317	69.15BCD	2.44CD	0.167BC	420AB
Magellan	77.20ABC	2.64ABC	0.205ABC	380BCDE
Maverick	79.80ABC	2.91ABC	0.235AB	354BCDE
PI398965	71.16BCD	3.25A	0.232AB	313DE
PI408255B	68.30BCD	2.84ABC	0.198ABC	365BCDE
PI417107	78.43ABC	3.13AB	0.243A	331CDE
PI423890C	77.35ABC	2.89ABC	0.224ABC	346BCDE
PI424614	56.95D	2.79ABC	0.159C	361BCDE
PI561.370	66.60CD	2.86ABC	0.192ABC	372BCDE
PI603171	76.45BC	3.01ABC	0.230AB	335CDE
PI603454	72.05BCD	2.58BCD	0.186ABC	402ABC
PI399027	78.91ABC	2.46CD	0.193ABC	412AB
S06-13640	71.950BCD	2.65ABC	0.193ABC	392BCDE
Min	56.95	1.933	0.159	311
Max	95.85	3.253	0.243	527
Mean	74.89	2.760	0.209	377
P-value	0.297	0.023	0.627	0.005
CV%	17.711	16.513	25.139	16.906
LSD	19.259	0.065	0.074	90.181

Table 2-7. Effect of soybean genotype on root morphological traits grown at Rollins Bottom in 2016.

No.	Genotype	AngLw	LRDLw	NLwR	AngUpR	LR	NUpR	ND	NS	TRDia	StemDia	Overall
1	4J105-3-4	22.400	7.267	15.533	2.133	8.500	4.400	2.667	2.933	6.333	9.667	2.667
2	CLOJ095-4-6	24.055	7.550	16.050	2.327	8.400	5.650	2.450	2.675	5.075	8.200	2.650
3	H10	21.800	8.450	16.400	4.250	8.133	5.950	1.850	2.400	5.450	10.400	2.750
4	H17	12.950	7.867	22.200	1.400	4.400	3.250	2.050	2.600	6.250	10.250	2.600
5	H7	24.050	7.800	22.200	2.400	6.000	4.350	2.700	2.600	6.150	10.050	3.550
6	IA3023	17.200	7.350	17.200	4.800	7.000	5.000	2.050	2.900	5.350	9.150	2.550
7	LG05-4466	15.650	8.050	22.400	0.667	7.133	3.467	2.200	2.700	4.600	8.800	2.550
8	LG05-4317	18.850	8.900	14.300	4.500	7.800	4.650	2.900	3.000	6.100	10.100	2.025
9	Magellan	15.650	7.650	12.650	1.800	6.200	4.950	2.400	2.650	6.450	10.450	2.250
10	Maverick	22.700	8.950	16.150	2.200	6.900	4.500	2.900	3.100	6.400	9.350	2.600
11	PI398965	21.750	9.850	17.400	3.067	7.950	5.700	2.300	2.700	6.150	10.150	2.800
12	PI408255B	18.900	7.900	20.450	3.800	7.400	7.800	1.900	1.900	5.350	8.900	3.350
13	PI417107	17.600	9.100	19.400	2.467	5.838	2.750	3.050	3.300	6.300	10.550	2.700
14	PI423890C	21.950	8.200	21.600	4.000	6.650	2.850	2.450	2.800	5.050	8.400	2.250
15	PI424614	24.525	8.150	16.700	2.733	8.525	3.800	1.800	2.150	5.300	7.750	2.600
16	PI561.370	16.400	7.250	14.650	2.850	7.013	3.600	1.550	2.500	5.400	9.850	2.200
17	PI603171	24.100	7.800	14.650	2.100	4.325	4.100	1.750	2.500	4.750	7.800	1.600
18	PI603454	19.700	8.250	14.000	1.550	7.850	2.800	2.350	2.650	6.250	9.500	2.300
19	PI399027	22.400	9.050	17.750	1.400	6.575	3.500	1.700	2.100	5.850	9.850	3.100
20	S06-13640	21.780	8.667	16.050	3.750	8.638	6.900	2.467	3.200	6.550	10.300	2.200
	Min	12.950	7.250	12.650	0.667	4.325	2.750	1.550	1.900	4.600	7.750	1.600
	Max	24.525	9.850	22.400	4.800	8.638	7.800	3.050	3.300	6.550	10.550	3.550
	Mean	20.221	8.203	17.387	2.710	7.061	4.498	2.274	2.668	5.755	9.473	2.565
	P-value	0.051	0.540	0.002	0.502	0.060	0.190	0.002	0.001	0.108	0.023	<0.0001
	CV%	25.471	17.275	20.329	78.194	27.246	52.072	23.043	15.039	17.199	13.330	17.014
	LSD	7.346	2.097	5.145	3.265	2.866	3.409	0.752	0.572	1.412	1.803	0.623

Table 2-8. Correlation matrix for root and shoot P traits of the 20 soybean genotypes grown at Rollins Bottom in 2016.

Trait	TapR Dia	Overall	Nod Size	NodDen	NADUpRoot	LatRoot DenAdUp	AngAdUp	NLowRoot	Lat RootDenLow	Ang Low	Shoot BM	Shoot P conc.	Shoot P cont.	PUE
StemDia	**0.435**	0.099	**0.250**	**0.249**	**0.328**	**0.259**	0.193	-0.176	0.076	-0.118	**0.220**	-0.006	0.173	0.035
	<0.000	0.387	0.026	0.028	0.004	0.023	0.109	0.126	0.515	0.302	0.055	0.957	0.127	0.759
TapRDia		0.120	0.021	0.100	-0.049	-0.136	0.005	-0.015	0.089	0.176	0.178	0.105	0.177	-0.128
		0.292	0.856	0.385	0.670	0.237	0.969	0.900	0.446	0.121	0.122	0.357	0.118	0.260
Overall			-0.014	**0.217**	0.042	-0.010	-0.114	**0.414**	**0.218**	-0.020	0.171	0.146	**0.214**	-0.122
			0.899	0.056	0.715	0.928	0.348	0.0002	0.061	0.859	0.137	0.198	0.059	0.284
NodSize				**0.695**	0.071	0.128	0.041	-0.018	0.148	**-0.286**	-0.055	-0.098	-0.136	0.062
				<0.0001	**0.538**	0.266	**0.737**	**0.876**	0.206	**0.011**	**0.636**	**0.389**	**0.233**	**0.587**
NodDen					0.063	**0.243**	0.056	-0.070	**0.271**	-0.014	0.099	0.028	0.050	-0.050
					0.586	0.035	0.651	0.551	0.019	0.906	0.395	0.806	0.665	0.662
NADUpRoot						**0.494**	**0.561**	**-0.306**	-0.186	0.048	0.177	-0.008	0.152	0.069
						<0.0001	<0.0001	0.008	0.115	0.675	0.128	0.942	0.187	0.554
LatRootDen AdUp							0.130	**-0.454**	0.221	0.115	-0.177	-0.037	-0.154	0.043
							0.300	<0.0001	0.068	0.332	0.136	0.758	0.192	0.717
LatRootDen AdUp							0.175	**-0.425**	0.153	0.108	-0.154	-0.026	-0.132	0.042
							0.159	0.0002	0.211	0.361	0.197	0.827	0.265	0.722
AngAdUp								-0.081	-0.061	0.155	0.064	-0.002	0.087	0.039
								0.511	0.629	0.201	0.602	0.985	0.472	0.746
NLowRoot									-0.040	-0.107	-0.013	0.065	0.006	-0.082
									0.735	0.354	0.913	0.572	0.961	0.481
LatRootDen Low										-0.082	0.009	0.015	-0.026	0.020
										0.482	0.940	0.898	0.826	0.867
AngLow											0.099	**0.311**	**0.244**	**-0.240**
											0.390	0.005	0.031	0.033
Shoot BM												0.001	**0.674**	0.026
												0.994	<0.0001	0.819
Shoot P conc.													**0.658**	**-0.968**
													<0.0001	<0.0001
Shoot P cont.														**-0.597**
														<0.0001

The first value in each cell represents Pearson correlation coefficient. The second value in each cell represents P value.

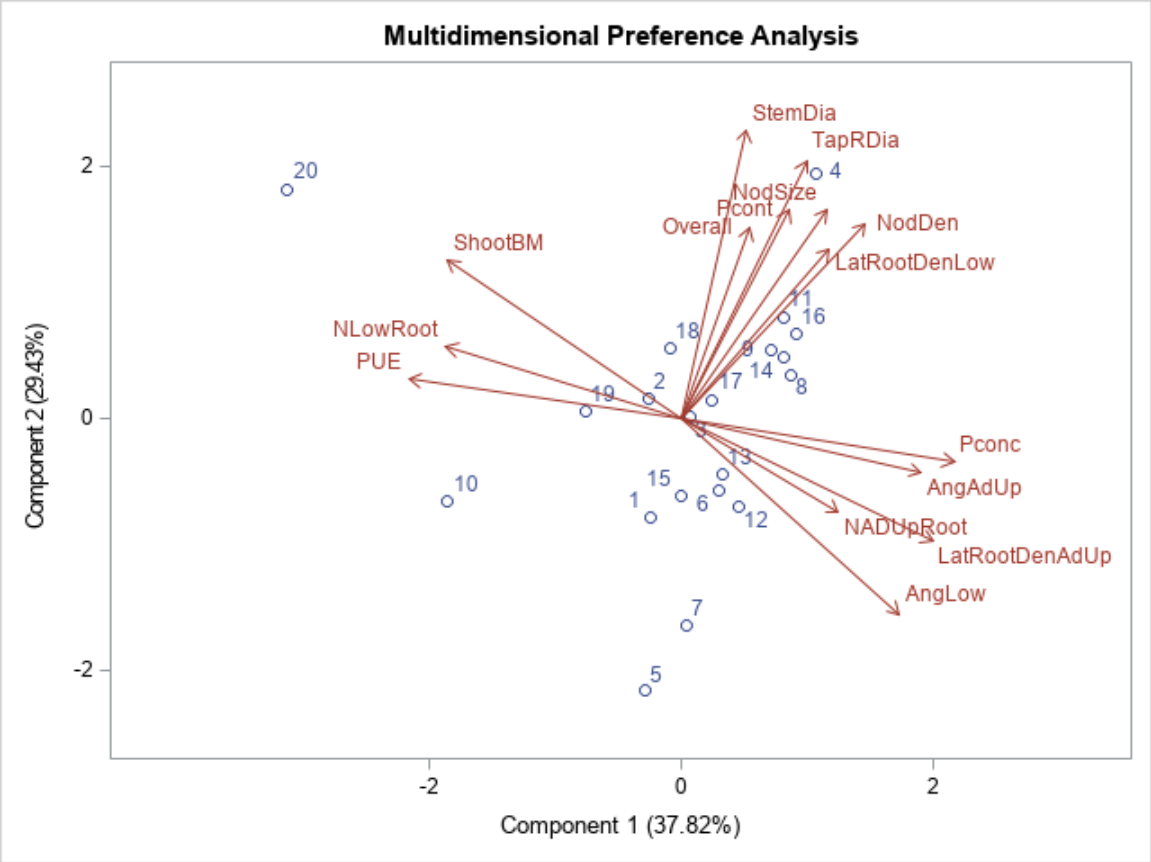

Figure 2-1. Principal component analysis of fifteen plant traits for 20 soybean genotypes grown at Rollins Bottom in 2016. Biplot vectors are trait factor loadings, whereas the position of each genotype is shown as number from 1 to 20 as specified in Table 2-7.

CHAPTER 3

EFFECT OF PHOSPHORUS AVAILABILITY ON PHOSPHORUS UPTAKE AND UTILIZATION EFFICIENCY OF SOYBEAN GENOTYPES

ABSTRACT

Soybean is one of the most valuable leguminous crops worldwide. Its growth and development are strongly influenced by P availability. Two sand culture experiments were conducted to examine the response of soybean genotypes to P availability. In the first experiment, two soybean genotypes (PI408255B and PI603454) were grown in six different P treatments (0, 0.1 0.3 0.5, 1.0, and 3.0 mM). In the second experiment, eight soybean genotypes were grown without supplemental P (0 P) and with 0.5 mM P. The growth responses of the two soybean genotypes to the six P treatments were very similar in that the 0.3 mM treatment dramatically improved growth over the 0 and 0.1 mM treatments and the impact of increasing P availability started to level off at the 0.5 mM P treatment. Low or no supplementation of the sand culture with P severally reduced growth of all soybean genotypes and increased root shoot ratios. Phosphorus treatments significantly influenced all measured traits significantly and the eight genotypes differed in shoot P content, shoot PUE, root P concentration, root P content, root PUE, whole plant P content and whole plant PUE. Except for whole plant PUE, significant genotype by P treatment interactions were observed for these eight genotypes. This study revealed genotypic variation not only for shoot P traits but also for root P traits and indicates that further studies on root P concentration and root content may hold promise to improve soybean performance under low P conditions.

INTRODUCTION

Soybean (*Glycine max* L. Merrill) is one of the most important crops worldwide with seed high protein and oil concentrations (Dornbos and Mullen, 1992; Medic *et al*., 2014). It is of high nutritive value not only for humans but also for animals and is widely used in different forms. Soybean also is important in developing countries as a substitute to relieve hunger and malnutrition (Alamu *et al*., 2018).

Compared to other crops, soybean acquires more P supply for its growth and especially for nodule development (Cassman *et al*., 1981). Thus, low P availability is of particular concern for soybean production and represents a major constraint for farmers growing soybean on low-P soil (Uhde-Stone, 2017). As estimated, 29% of the global cropland area is P deficient and 71% has surplus P (MacDonald *et al*., 2011). To satisfy crop production, P fertilizers often are applied in excess (MacDonald *et al*., 2011) which leads major concerns associated with depletion of P resource reserves and environmental pollution. Only 10 – 20% P applied is used by plants the rest is fixed in soil and some can be lost through run off which causes pollution of rivers, lakes, and ocean (Mclaughlin *et al*., 1988; Sharpley *et al*., 2003). Phosphorus reserves are limited and currently are predicted to be depleted in about 500 – 600 years (Sharma *et al.,* 2013). Thus, efforts to improve the efficient use of available P resources are critical. Breeding P efficient plants is considered an economic and environmentally friendly way to help mitigate problems of environmental pollution and finite rock phosphate reserves.

Efficiency of P use can be assessed in several ways. Depending on the definition, enhanced efficiency of P use can be achieved by improving uptake and/or

utilization efficiency (Batten, 1992; Osborne and Rengel, 2002). High PUE can be defined as the ability of a plant to produce high biomass with low amounts of P presented in its tissue. This has also been referred to as the internal PUE and can be improved by translocation of P within the plant or increase the root to shoot ratio (Hammond *et al*., 2004). On the other hand, P uptake efficiency often refers to the ability of a plant to extract P from the environment to achieve optimum yields in low P available conditions (Föhse *et al*., 1991). Phosphorus uptake efficient plants can enhance access to non-labile P through numerous mechanisms, including root system modifications, exudation of organic acids and/or phosphatase enzymes, and through symbiotic relationships with mycorrhizal fungi (Hedley *et al*., 1982; Tarafdar, 1987; Dinkelaker *et al*., 1989; Hocking, 2001; Begum *et al*., 2019).

Soybean genotypes differ in their ability to acquire P from P-limited as well as P sufficient soil (Pan *et al*., 2008; Zhou *et al.*, 2016). Besides increased root growth and changing root architecture, altering root growth angle, and production of adventitious roots, have all been reported to be the root traits that are necessary for adaptation of soybean to low P conditions (Zhao *et al*., 2004). Moreover, root hairs, root transporters, root exudate, association with AMF, are all involved in improved P uptake in plants (Smith and Read, 2008). In the study described in Chapter 2 we have identified soybean genotypes that differ in P-related traits when grown in the field in P-sufficient conditions. The information gained from those studies will be useful to develop more P efficient soybean cultivars for P-sufficient environments, but it may not apply to P-deficient environments. Although numerous studies have been conducted on the responses of soybean to low P conditions, they have primarily been

focused on Chinese germplasm (Zhao *et al*., 2021) on P uptake related traits and to a lesser extent on PUE. The objectives of this study were to (i) examine soybean growth responses to a range of P availability treatments (ii) evaluate soybean genotypes variability in terms of P uptake, and PUE at the whole plant level using a system that facilitates quantitative recovery of root systems.

MATERIALS AND METHODS

Two pot experiments were carried out to study the response of soybean genotypes to different P levels. The first experiment (Experiment 1) was conducted in a greenhouse at the University of Missouri, Columbia, MO, to determine the growth response curve of soybean to six different levels of P availability. Based on results from Experiment 1, two levels of P availability were selected to study the response of eight genotypes to low-P and P-sufficient conditions.

Experiment 1: Soybean responses to six levels of P availability under greenhouse conditions.

Two soybean genotypes were selected for a greenhouse experiment based on results from studies described in Chapter 2. PI603454 had high biomass, low P content, high PUE, and a root Overall complexity score of 2.3. PI408255B had high P content, low PUE and a root Overall complexity score of 3.35. The two genotypes were grown in a greenhouse at the University of Missouri in Columbia in 7.6-L pots filled with coarse sand where six different P-availability treatments were imposed. Coarse sand was analyzed by the Soil Testing and Plant Diagnostics Services, University of Missouri, USA. Bray-I P test revealed very low levels of P (from 6.73-10.09 kg ha^{-1}) and therefore the sand was considered deficient in P and suitable for

the experiment. Conditions in the greenhouse were as follows: temperature 25/20°C (day/night), relative humidity ~75%, average daytime photosynthetically active radiation between 800 and 1000 µmol m^{-2} s^{-1}, and a photoperiod of 14/10 h day/night. The experiment was arranged in a randomized complete block design with four replications. Three seeds of each genotype were sown on 1 February 2017 and pots were thinned to one plant at the first true leaf stage. A drip system was used to irrigate the plants with Hoagland solution as modified by Johnson *et al*. (1957): 800 mM $CaCl_2.2H_2O$, 150 mM K_2SO_4, 200 mM $MgSO_4.7H_2O$, 1M NH_4NO_3, 800 mM KCl, 12.5 mM FeEDTA, 2.3 µM H_3BO_3, 0.9 µM $MnSO_4.H_2O$ 0.6 µM $ZnSO_4.7H_2O$, 0.1 µM $NaMoO_4 \cdot 2H_2O$, 0.11 µM $NiCl_2.6H_2O$, 0.01 µM $CoCl_2.6H_2O$, 0.15 µM $CuSO_4.5H_2O$. The six different phosphorus levels of 0, 0.1, 0.3, 0.5, 1.0, and 3.0 mM were generated by adding appropriate amounts of 200 mM KH_2PO_4 and 200 mM NaH_2PO_4 for each treatment. The pH of the nutrient solutions was checked daily and maintained at pH 6.0. New nutrient solutions were made every 5 days.

Treatment effects were assessed by measurements of plant height, developmental stage, chlorophyll content, biomass production and partitioning, and nutrient analyses. Specifically, plant height (cm) was measured from the sand surface to the apical meristem. Total chlorophyll content was measured using a SPAD-502 meter at 43, 56 and 70 DAP. At mid pod filling (R5.5) growth stages, plant stems were cut 2 cm above the sand surface, and leaves, pods, and stems were separated. The total leaf area was measured using a LI-3100 leaf area meter (LICOR, NE). Roots were washed carefully to remove all sand. All samples were dried in the oven at 65°C until constant weight, and the dry weight of each plant fraction was determined

separately. To calculate the dry matter. Dried and weighed samples were ground with a cyclone mill (Cyclotech, Foss North America) through a 1 mm screen and sent for analysis by a Spectro ARCOS ICP-OES at the Agriculture Diagnostic Lab at the University of Arkansas.

Experiment 2: Responses of eight soybean genotypes to two levels of P availability.

Eight genotypes (PI424614, PI603454, LG054317, 4J05-3-4, IA3023, H7, H10, and H17) were selected for this study based on results described in Chapter 2. Based on results from Experiment 1, two P treatments, namely 0 P (no P fertilization) and 0.5 mM P were included in this experiment. Pots (18.9 L) were filled with 28 kg of coarse sand (same as for Experiment 1) and placed in the field at the Bradford Research Center in Columbia, MO. Five seeds were sowed in each pot on 1st July 2017. Ten days after the seeds germinated, pots were thinned to two healthy plants which were grown until sampling at mid pod fill (R5.5). Plants were watered through a drip irrigation system with the same nutrient solution as described for Experiment 1, with the 0 P treatment receiving solution without P and the 0.5 P treatment receiving solution containing 0.5 mM P. Pots were watered to excess every day, and after each rainy day, all pots were flushed with DI water before applying nutrient solution like every other day.

Plant height and biomass sampling was conducted as described for Experiment 1. Abscised leaves and petioles were carefully gathered each week and dried and weighed in the same manner as all other plant fractions. Weights of the abscised material were added to the appropriate dry sample weights. After dry weight

determinations, all plant fractions were ground separately with a cyclone mill through a 1 mm sieve and sent for nutrient analyses by a Spectro ARCOS ICP-OES at the Agriculture Diagnostics Lab at the University of Arkansas.

Shoot and root P content were calculated based on the dry weights of the relevant tissues and the corresponding tissue P concentrations. Root P acquisition efficiency (RPAE) was calculated as plant P content divided by root dry weight. The P efficiency ratio (PE %) is an expression of the relative shoot growth calculated as the percentage of shoot DM production under no P to that under adequate P supply (Ozturk *et al*., 2005).

Statistical analysis

The following variables were measured or calculated: Shoot biomass or dry matter (DM), plant height, total leaf area, root to shoot ratio, shoot and root P concentration, shoot and root P uptake, PUE, RPAE, P efficiency ratio. Analysis of Variance (ANOVA) was conducted for all data using PROC GLIMMIX by SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA) to determine genotype, treatment, and interaction effects. Genotype and P treatment were treated as fixed effects while replication and interaction were treated as random effects. Mean separation was performed by Fisher's least significant difference (LSD) test with $\alpha = 0.05$. Correlation analyses were conducted using PROC CORR in SAS. Graphs were produced using SigmaPlot (SigmaPlot, Systat Software, Inc, Richmond, CA, USA).

RESULTS

Experiment 1: Responses of two soybean genotypes to increasing P availability.

PI603454 and PI408255B were grown in coarse sand and irrigated with modified Hoagland solution with either 0, 0.1, 0.3, 0.5, 1.0, or 3.0 mM P. Although some differences between genotypes were observed in the actual amounts of some plant fractions (*e.g.* leaf, stem, and root dry matter) at some P levels, both genotypes generally exhibited the same response pattern to increasing P availability. Plant growth as measured by plant height, number of main stem nodes, total plant, leaf, stem, pod, and root dry matter of both genotypes was severely impaired in the 0 and 0.1 mM P treatments and increased dramatically in response to the 0.3 mM P treatment (Figure 3-1). The impact of a further increase in P supply in the 0.5 mM treatment generally only resulted in a limited enhancement of growth and a further increase in P availability did not significantly enhance growth, and, for some traits, the 3.0 mM P treatment impaired growth.

Largely independent of P treatment, the root to shoot ratio of PI408255B was greater than that of PI603454 which contrasted with the generally greater pod dry matter of PI603454. These observations are consistent with the earlier maturity of PI603454 which thus shifted allocation to pods as opposed to roots earlier.

Shoot and root P concentration and content increased significantly with increasing P availability, reaching a maximum in the 3.0 mM treatment (Figure 3-2). While not different in 0, 0.3, 0.5, and 1.0 mM P treatments, shoot and root P concentrations were greater in PI603454 than in PI408255B in the 3.0 mM P treatment. In the 3.0 mM P treatment, root P concentrations were considerably greater in root tissue compared to shoot

tissue, but the opposite trend was observed for all other treatments. Shoot PUE decreased with increasing P availability (Figure 3-2E), which is consistent with a strong negative correlation with shoot P concentration.

Root P acquisition efficiency (RPAE) largely remained stable for 0 to 1.0 mM P treatments and was much greater in the 3.0 mM P treatment. When plants were grown with solution containing 3 mM, RPAE and P content of PI603454 was about 2.6 and 1.2 times, respectively, greater than that of PI408255B. However, while the genotypes by P treatment interaction was significant for RPAE, it was not for shoot and root P content.

Experiment 2: Impact of low P and sufficient P supply on growth and P traits of eight soybean genotypes.

Plant growth, biomass allocation, and several P traits were determined for eight soybean genotypes that were grown in the field in pots filled with coarse sand and irrigated with modified Hoagland solution containing no P or 0.5 mM P. Analysis of variance revealed strong genotype, P treatment and genotype by P treatment interaction effects for all examined traits, except for shoot biomass for which the genotype by environment interaction effect was just missed the 0.05 cut off (P = 0.068; Table 3-1). Growth of all genotypes in the -P treatment was severely stunted which resulted in dramatic differences in biomass accumulation between the two P treatments (Figure 3-3). While plant height differed by almost 2-fold between the two treatments and across all genotypes, total biomass accumulation as well as biomass of the different plant fractions was 32 to 78-fold greater in the +P treatment than the -P treatment. The average plant height of ranged from 18.8 cm to 24.1 cm in -P treatment and from 38.6 cm to 43.5 cm in the +P treatment.

The average shoot biomass in the +P treatment varied from 31.69 g for 4J05-3-4 to 42.35 g for H10, and from 0.43 g for H7 to 0.83 g for 4J05-3-4 in the -P treatment. Averaged across all eight genotypes, shoot biomass was reduced from 37.64 g for the +P to 0.62 g for the -P treatment (nearly 98%) and root biomass was reduced by 93% from 11.83 g in the +P treatment to 0.75 g in the -P treatment. On average across all genotypes, the root to shoot ratio was 3.8-fold greater in the -P treatment than the +P treatment, indicating prioritization of resource allocation for root growth under low P conditions.

The three genotypes selected under low P conditions in Africa, H7, H10, and H17 produced high biomass under in +P conditions, but because they are not adapted to the Midwestern photoperiod, they flowered late and thus did not produce many pods by the time plants were sampled.

P deficiency significantly influenced P accumulation in shoots and roots of all genotypes (Table 3-2). P accumulation in the shoot ranged from 0.24 mg $plant^{-1}$ (H7) to 0.73 mg $plant^{-1}$ (4J05-3-4) in the -P treatment, and from 60.15 mg $plant^{-1}$ (H7) to 82.86 mg $plant^{-1}$ (H17) in the +P treatment.

Root P concentrations ranged from 0.40 g kg^{-1} (PI424614 and IA3023) to 0.58 g kg^{-1} (PI603454) in the -P treatment, and from 1.10 g kg^{-1} (PI424614 and IA3023) to 2.00 g kg^{-1} (H7) for plants grown in the +P treatment. These concentrations are lower than those found in shoot tissue which ranged from 0.59 g kg^{-1} (H7) to 0.92 g kg^{-1} (LG05-4317) in -P treatments and from 1.69 g kg^{-1} (PI424614) to 2.18 g kg^{-1} (4J05-3-4) in +P treatments. On average for all genotypes, root P content decreased from 17.27 mg $plant^{-1}$ the +P treatment to 0.35 mg $plant^{-1}$ in the -P treatment (Table 3-3). The maximum and minimum

root P content in the +P treatment were recorded in for H17 (25.54 mg plant^{-1}) and IA3023 (10.21 mg plant^{-1}), respectively. In the -P treatment, the lowest amount of P accumulated in roots was in H17 (0.242 mg plant^{-1}) and the highest amount was found in PI603454 (0.502 mg plant^{-1}).

On average across all genotypes, total uptake of P per plant increased from 0.85 mg plant^{-1} in the -P treatments to 86.9 mg plant^{-1} in plants grown in +P treatments. The genotypes H10 (105.49 mg plant^{-1}) and H17 (108.39 mg plant^{-1}) showed maximum total P accumulation in +P, while PI603454 and 4J05-3-4 recorded the highest total P accumulations (1.142 and 1.182 mg plant^{-1}, respectively) in the plant in the -P treatment.

Relationship between plant growth, development, and plant P status

Trait correlations for plants grown in -P and +P treatments are shown in Tables 3-4 and 3-5, respectively. In general, relationship between the studied traits were consistent at both P levels. Shoot biomass and root biomass production were positively correlated in -P and +P treatments (r = 0.645 and 0.539, respectively) and root and shoot P content were positively correlated with root and shoot biomass. Phosphorus use efficiency was positively correlated with BM only in the -P treatment (r = 0.581), but highly negatively correlated with shoot P concentration in both P treatments (r = -0.978 in -P treatment and r = -0.845 in +P treatment). Root P acquisition efficiency was negatively correlated with PUE in both treatments (r = -0.564 in +P treatment and -0.588 in -P treatment). Interestingly, RPAE was positively correlated with shoot P content in both P treatments (r = 0.726 in both P treatments), but the correlations of RPAE with root P content were not significant in either P treatment. The root shoot ratio (RSR) was positively correlated with root weight and but negatively correlated with RPAE.

DISCUSSION

Plants absorb phosphorus in soil in the orthophosphoric forms ($H_2PO_4^-$ or HPO_4^{2-}). Depending on soil pH, these anions easily react with soil cations such as calcium, magnesium in Alkaline soil, or iron, and aluminum in Acid soil to produce various insolubility phosphate compounds which plants cannot absorb. Soybean exhibits genetic variation for P efficiency with respected to yield, P accumulation potentials and root physiological characteristics (Zhou *et al*., 2016). Genetic variability for P uptake, utilization and translocation has been reported by a great number of researchers (Gerloff 1963; Vose, 1963, 1990; Elliot and Laüchli, 1985; Wild *et al*., 1987; Baligar and Duncan, 1990; Noordwijk *et al*., 1990; Gahoonia *et al*., 1992; Ozturk *et al*., 2005). Consistent with these studies, the absence of P addition in the -P treatment severely inhibited growth compared to the P-supplemented treatment.

The root to shoot ratio is an important trait for P use efficiency (Hermans *et al*., 2006), and is strongly influenced by soil P status. Usually under low P conditions, an increase in the proportion of roots produced is commonly observed and has been documented for many species (*e.g*. Atkinson, 1973; Bohm, 1979; Anghinoni and Barber, 1980; Hayes and Ludecke, 1981; Pereira and Bliss, 1987; Biddinger *et al*., 1998). Indeed, much greater root to shoot ratios were found in the -P than the +P treatments in all genotypes in this study (Figure 3-4). This is an adaptation mechanism to improve P uptake when it is limiting growth (Lynch, 1995). Under P deficient conditions, plant root to shoot dry weight ratio also tends increase because of preferential accumulation of carbohydrates in roots (Cakmak *et al*., 1994; Hermans *et al*., 2006).

The physiological PUE of soybean genotypes in Experiments 1 and 2 were calculated as method of Fageria *et al*. (2013), and are presented in Figure 3-2, Figure 3-4, respectively. As expected, shoot PUE decreased with increasing P application and shoot and root PUE were significantly higher in the -P treatment compared to the +P treatment.

Physiological PUE was reliably and strongly negatively correlated with shoot P concentration Tables 3-4, 3-5) which is consistent with results reported for soybean in other chapters and by Furlani *et al*. (2002). Additionally, shoot PUE was negatively correlated with the root P acquisition efficiency.

Critical for genetic improvement of soybean PUE is the fact that genotypic variation in PUE was observed at the shoot level and the whole plant level. Thus, it should be possible to leverage genotypes with high PUE (*e.g*. PI424614) for germplasm improvement efforts. For genetic studies genotypes with low PUE (*e.g*. 4J05-3-4) can play an important role when paired with a high PUE genotype as parents for biparental mapping populations. Similarly, genotypes from that fall on the phenotypic tails of a population also are useful for comparative physiological studies.

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TABLES AND FIGURES

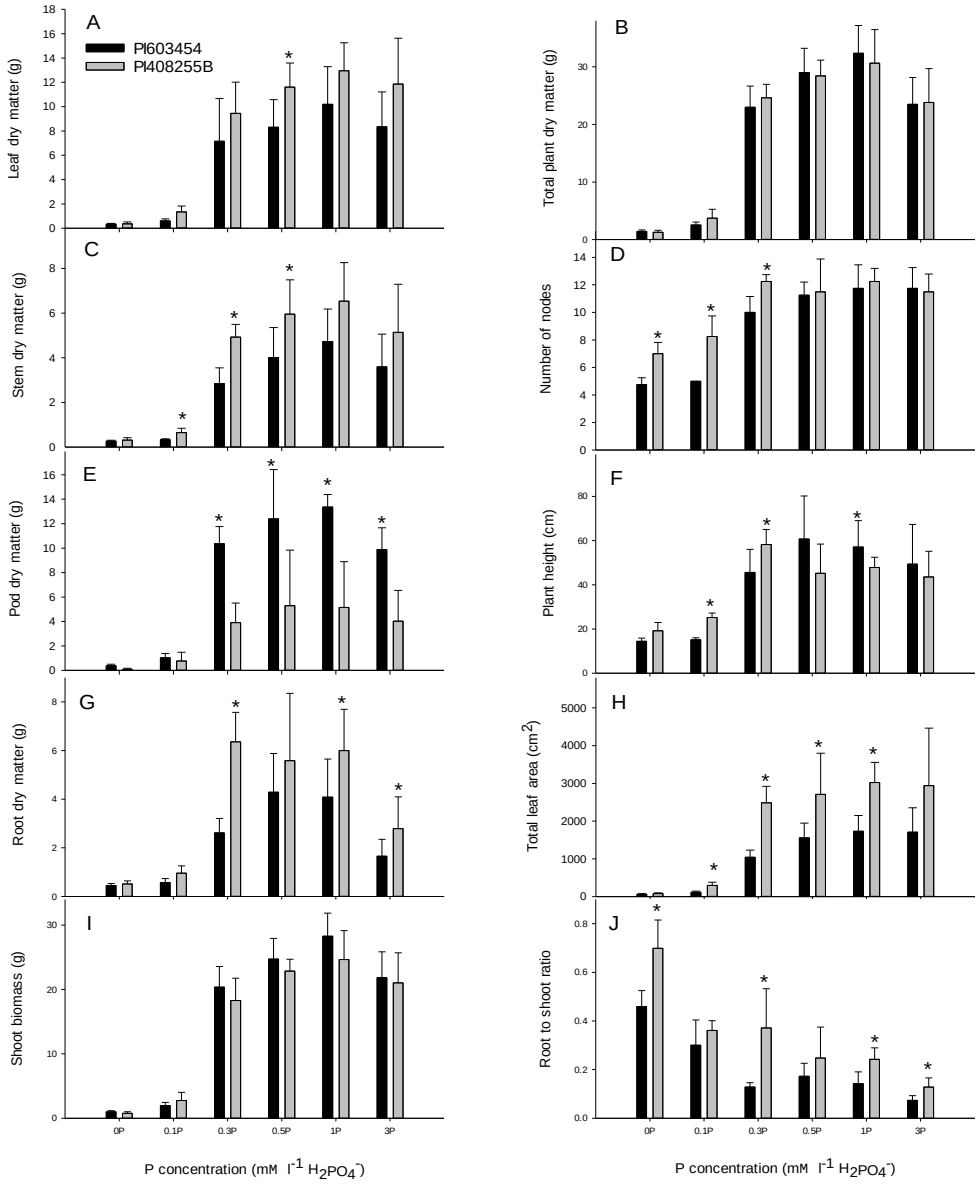

Figure 3-1. Response of soybean genotypes PI603454 and PI408255B to different P availability under greenhouse conditions. Data are means of four replicates and bars represent standard errors. Asterisks indicate significant differences between genotypes at α = 0.05.

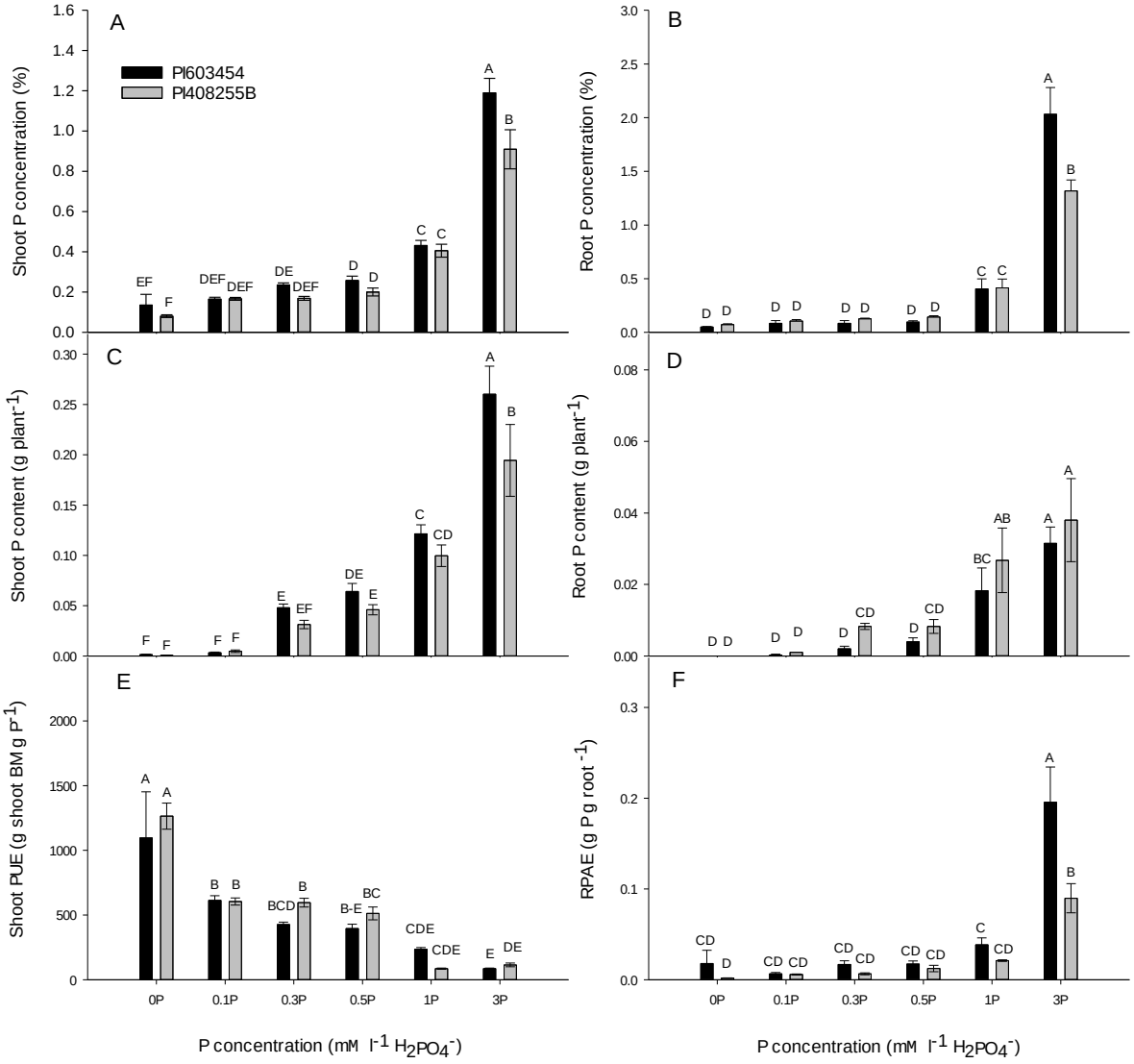

Figure 3-2. Phosphorus concentration, content, shoot P use efficiency (PUE) and root P acquisition efficiency (RPAE) of PI603454 and PI408255B under different P availability under greenhouse conditions. Data are means of four replicates and bars represent standard errors. Bars with different letters are significantly different at $P < 0.05$.

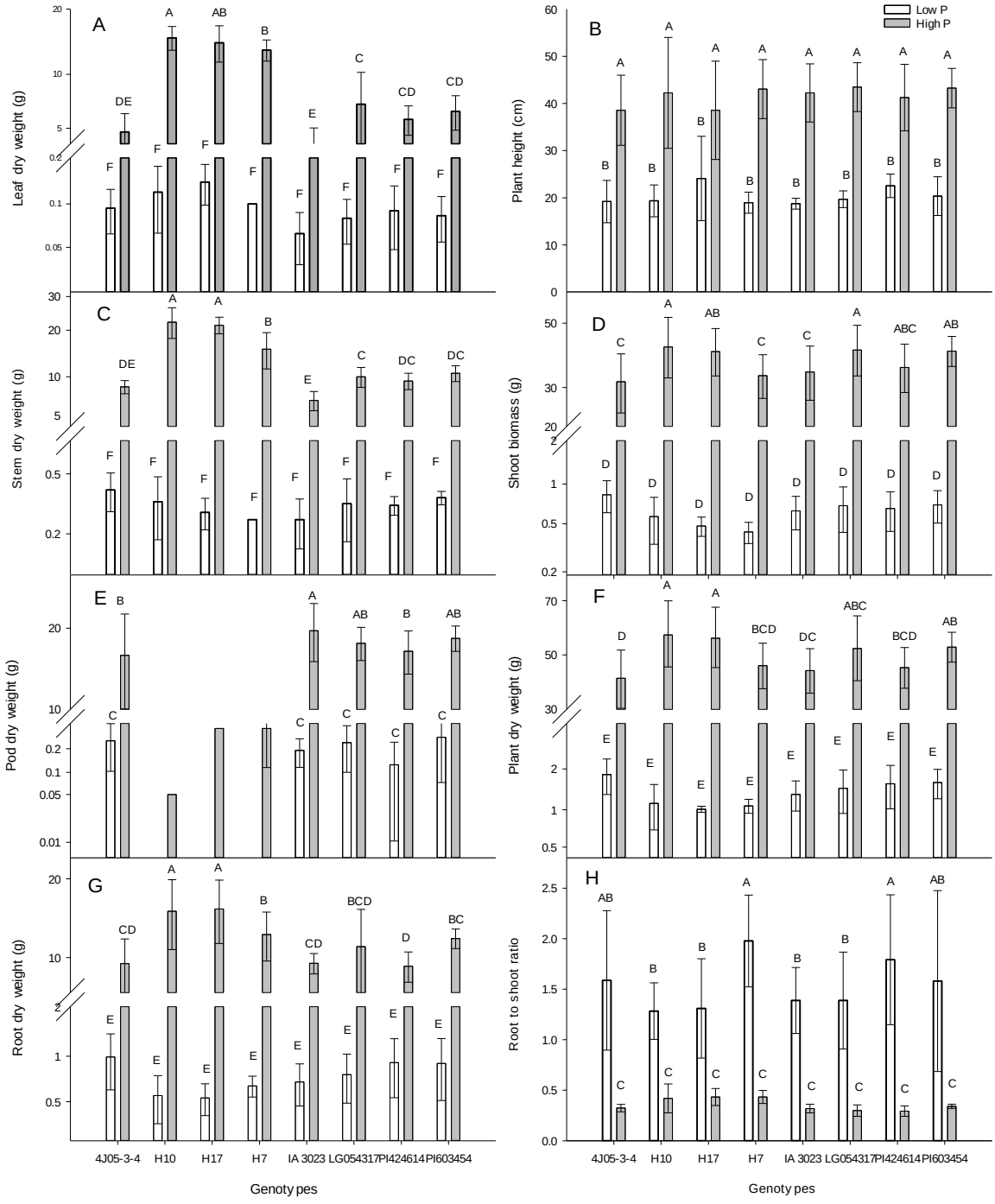

Figure 3-3. Growth responses of soybean genotypes to low and sufficient P conditions. Data are means of five replications and bars represent standard errors. Bars with different letters are significantly different from each other at α = 0.05.

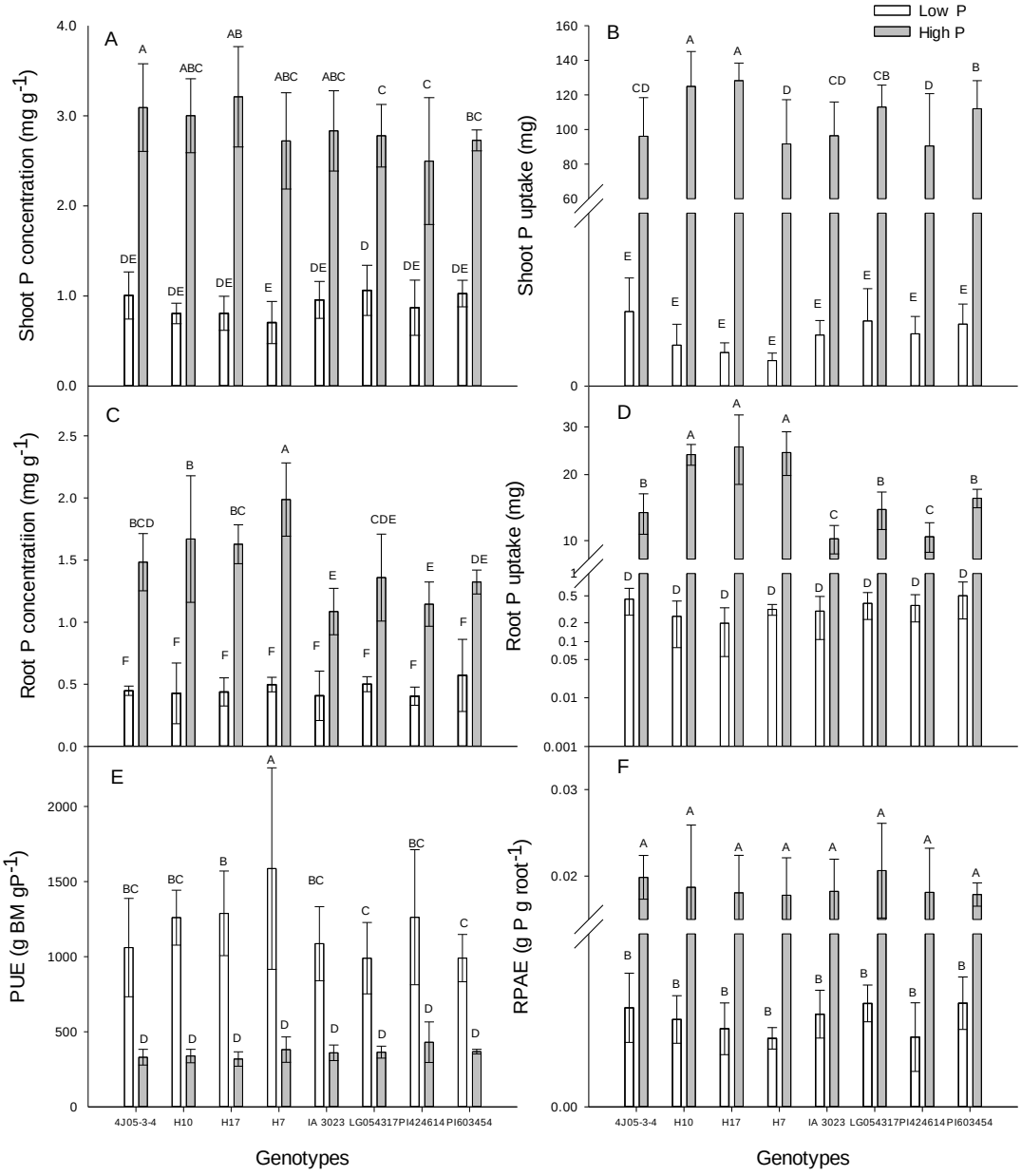

Figure 3-4. Shoot and root P concentration, shoot and root P uptake, shoot PUE and root P uptake efficiency of soybean genotypes under low and sufficient P conditions. Data are means of five replications and bars represent standard errors. Bars with different letters are significantly different from each other at $\alpha = 0.05$.

Table 3-1. Analysis of variance results (P values) for stem, leaf, pod, root, shoot, and total plant dry matter, total leaf area (TLA), specific leaf area (SLA), and root to shoot ratio (RSR) of eight soybean genotypes grown in pots without (-P) or with (+P) fertilizer.

Source	Dry matter												TLA		SLA		RSR	
	Stem		Leaf		Pod		Root		Shoot		Plant							
	- P	+ P	-P	+ P	-P	+ P	-P	+ P	- P	+ P	- P	+ P	- P	+ P	- P	+ P	-P	+ P
Genotype (G)	<0.0001		<0.0001		<0.0001		0.0036		0.0430		0.0013		<0.0001		<0.0001		<0.0001	
Treatment (T)	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
G x T	<0.0001		<0.0001		<0.0001		0.0007		0.0678		0.0004		<0.0001		<0.0001		<0.0001	

Table 3-2. Means of shoot P concentration, shoot P content, and shoot PUE, and associated analysis of variance results (P-values), for eight soybean genotypes grown in pots without (-P) or with (+P) fertilizer.

	Shoot P concentration ($g\ kg^{-1}$)		Shoot P uptake ($mg\ plant^{-1}$)		Shoot PUE ($gBM\ gP^{-1}$)	
Genotypes	- P	+ P	- P	+ P	- P	+P
4J05-3-4	0.8246	2.1828	0.73	65.07	1396	485
H10	0.6249	1.9829	0.37	81.57	1621	519
H17	0.7347	2.0877	0.35	82.86	1523	494
H7	0.5917	1.8109	0.24	60.15	2278	560
IA3023	0.8071	1.9313	0.48	64.03	1449	538
LG054317	0.9222	1.7490	0.66	71.27	1261	581
PI424614	0.6303	1.6989	0.44	60.40	1751	604
PI603454	0.9099	1.7432	0.64	71.58	1156	574
P values						
Genotype (G)	0.3265		0.0006		0.0448	
Treatment (T)	<0.0001		<0.0001		<0.0001	
G x T	0.0056		0.0006		0.0040	

Table 3-3. Means of root P concentration, root P content, root PUE and plant P concentration, plant P content and plant PUE, and associated analysis of variance results (P-values), for eight soybean genotypes grown in pots without (-P) or with (+P) fertilizer.

	Root P concentration		Root P uptake		Root PUE		Plant P concentration		Plant P uptake		Plant PUE	
	g kg^{-1}		mg $plant^{-1}$		g root dry weight/ root P content		g kg^{-1}		mg $plant^{-1}$		g plant dry weight/ plant P content	
Genotypes	-P	+ P	- P	+ P	- P	+ P	- P	+ P	- P	+ P	-P	+P
4J05-3-4	0.44	1.50	0.448	13.69	2246	687	0.638	2.017	1.182	78.759	1677	518
H10	0.42	1.70	0.251	23.92	6874	634	0.523	1.887	0.621	105.492	2131	547
H17	0.47	1.60	0.242	25.54	2443	619	0.622	1.973	0.625	108.394	1691	518
H7	0.48	2.00	0.318	24.34	2034	513	0.528	1.860	0.558	084.491	1990	542
IA 3023	0.40	1.10	0.3	10.21	4348	944	0.588	1.731	0.775	74.236	1825	591
LG054317	0.50	1.40	0.39	14.13	2026	755	0.698	1.664	1.046	85.399	1495	612
PI424614	0.40	1.10	0.363	10.47	2550	888	0.515	1.577	0.829	070.871	1991	645
PI603454	0.58	1.30	0.502	15.87	2023	758	0.717	1.648	1.142	87.452	1449	608
P values												
Genotype (G)	0.0007		<0.0001		0.1106		0.0877		<0.0001		0.0497	
Treatment (T)	<0.0001		<0.0001		<0.0012		<0.0001		<0.0001		<0.0001	
G x T	0.002		<0.0001		0.0159		0.0029		<0.0001		0.2347	

Table 3-4. Correlations among shoot biomass, root biomass, root to shoot ratio, total leaf area, and shoot P concentration, root P concentration, shoot P content, root P content, P use efficiency (PUE), and root P acquisition efficiency (RPAE) for plants grown in the -P treatment (* $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$).

Traits	Shoot biomass	Root biomass	Root: Shoot ratio	Total leaf area	Shoot P concentration	Root P concentration	Shoot P content	Root P content	PUE	RPAE
Shoot biomass										
Root biomass	**0.645*****									
Root: Shoot ratio	0.057	**0.771*****								
Total leaf area	0.458	**0.798*****	**0.633*****							
Shoot P concentration	**-0.616*****	-0.308	-0.017	0.066						
Root P concentration	-0.189	-0.006	0.177	0.327	0.277					
Shoot P content	**0.637*****	**0.529****	0.085	**0.550****	0.180	0.077				
Root P content	**0.388***	**0.789*****	**0.736*****	**0.850*****	-0.119	**0.587*****	**0.421****			
PUE	**0.581*****	0.222	-0.060	-0.002	**-0.978*****	-0.285	-0.241	0.042		
RPAE	**-0.398***	**-0.718*****	**-0.671*****	**-0.379***	**0.652*****	**0.343****	**0.726*****	0.115	**-0.588****	

Table 3-5. Correlations among shoot biomass, root biomass, root to shoot ratio, total leaf area, and shoot P concentration root P concentration, shoot P content, root P content, P use efficiency (PUE), and root P acquisition efficiency (RPAE) for plants grown in the +P treatment (* P < 0.05; ** P < 0.01 and *** P < 0.001).

Traits	Shoot biomass	Root biomass	Root: Shoot ratio	Total leaf area	Shoot P concentration	Root P concentration	Shoot P content	Root P content	PUE	RPAE
Shoot biomass										
Root biomass	**0.539****									
Root: Shoot ratio	-0.301	**0.512****								
Total leaf area	-0.003	0.090	-0.094							
Shoot P concentration	0.184	0.247	-0.256	0.094						
Root P concentration	0.178	0.201	0.025	0.187	**0.654*****					
Shoot P content	**0.729*****	**0.456****	**-0.347***	-0.019	**0.774*****	0.187				
Root P content	**0.479****	**0.827*****	**0.373****	0.236	0.236	**0.654*****	**0.412***			
PUE	-0.140	-0.148	0.355	-0.247	**-0.845*****	-0.105	**-0.611*****	-0.129		
RPAE	**0.411****	-0.114	**-0.661*****	-0.033	**0.659*****	**0.429****	**0.726*****	0.139	**-0.564*****	

CHAPTER 4

EVALUATION OF SOYBEAN GENOTYPES FOR PHOSPHORUS USE EFFICIENCY UNDER FIELD CONDITIONS

ABSTRACT

Phosphorus (P) is an essential macronutrient that limits crop productivity, including that of soybean (*Glycine max* L. Merrill), in many environments. Phosphorus uptake ability and P use efficiency (PUE) of a crop critically influence its productivity. Field studies were conducted to explore the genetic variation in shoot P concentration, shoot P content, and PUE, and plant response to different P levels. In the first year, 119 diverse soybean genotypes, including soybean nested association mapping (SoyNAM) parents, obsolete cultivars, and plant introductions were grown in a randomized complete block design with five replications under two P treatments (Zero P fertilizer *vs*. 112 kg ha^{-1} MAP) arranged as split plots. Genotype and P treatment strongly influenced shoot P content and P concentration, but no interactions between genotype and P treatment were detected. Shoot P concentrations ranged from 1.65 g kg^{-1} to 2.89 g kg^{-1} in Zero P and from 1.88 g kg^{-1} to 3.25 g kg^{-1} in the Plus P treatment. Shoot P content was greater in Plus P compared to Zero P treatments and ranged from 0.036 g $plant^{-1}$ to 0.118 g $plant^{-1}$ in Zero P and from 0.054 g $plant^{-1}$ to 0.142 g $plant^{-1}$ in the Plus P treatment. Shoot P content, an indication of a genotype's ability to take up P from the soil, and P concentration as an indicator of P use efficiency varied significantly among the genotypes characterized in this study.

In the second year, a subset of 20 genotypes were grown to test the consistency of the relationship between P accumulation and biomass production across years as well as

relationships with yield. Biomass and seed yield were increased by P fertilization and positively correlated with shoot P accumulation. Examination of arbuscular mycorrhizal fungi (AMF) colonization of roots for five of the 20 genotypes revealed very high colonization rates in both the P fertilized and unfertilized treatments as well as in all genotypes (> 85%). The AMF colonization rate was not related to any of the measured plant traits. The 20 genotypes were classified into four groups, efficient and responsive, efficient, and non-responsive, inefficient, and responsive, and inefficient and non-responsive based on relationships of PUE and biomass production. Genotypes in these different categories can be leveraged for genetic and physiological studies. For germplasm improvement efforts, genotypes falling into the efficient quadrants are of particular interest.

INTRODUCTION

Among different macronutrients, phosphorus (P) plays a vital role in a plant's growth and development. However, its availability in soil solution is very low, often less than 10 µM (Schachtman *et al.,* 1998). It constitutes less than 0.1 percent of total soil P (Khan *et al.,* 2009). With low P availability, the plant will not absorb enough P to increase vegetative growth (Gardner *et al*., 1985). The suboptimal P level requires an extra application of P fertilizer to optimize crop production (Ramaekers *et al.,* 2010). Applied P can be adsorbed to soil particles, precipitate with soil mineral such as Al, Fe or Ca, depending on soil pH, immobilized by soil microorganisms, or remain in the soil solution (Tisdale & Nelson, 1975; Sylvia *et al.,* 2005). It is estimated that > 80% of applied P is fixed in soil (Gill *et al.,* 1994; Trolove *et al*., 2003; Vance *et al*., 2003), or converted to organic forms (Holford 1997), and becomes unavailable to plants. The demand for P fertilizer for crop production has increased globally which leads to the depletion of this nonrenewable P resource. Thus, improvement P use efficiency by either management practices or breeding programs are needed to sustain agricultural production.

To adapt to low P availability, plants exhibit different mechanisms. Changing the physiological processes in root systems is one of the important mechanisms. It is observed that plant roots can either increase root hair length and density (Bates and Lynch, 1996; Ma *et al*., 2001), and the prevalence of lateral roots (Zhang *et al*., 2009), or symbiosis with mycorrhizal fungi (Smith *et al*., 1999), or exudes P mobilizing compounds such as protons, organic acids, and phosphatases (Ae *et al.,* 1993; Keerthisinghe, 1998; Neumann *et al*., 1999; Ryan *et al.,* 2001; Hinsinger, 2001). All these processes are involved with root morphology and architecture. Because root characteristics will determine the distribution

of roots in soil, these characteristics determine the ability of plant roots to explore and exploit P resources.

In soil, P is immobile, but it is mobile in plants. Under P deficiency, plant roots are poorly developed. This can be seen in the older leaf as the first symptom of P deficient plants. For example, older tomato leaves will turn purple when exposed to P deficient conditions. But unlike leaves, roots are less effective in mobilizing P to the rest of the plant through programed organ senescence (Snapp and Lynch, 1996). That why the cost of P for root growth is greater than that for leaf growth. Therefore, plants with optimal root systems for P acquisition not only improve P uptake at a minimum carbon cost but also maximize the value of P gained regarding the relative value of the resources required for root growth (Lynch and Ho, 2005). In addition, plants exhibit genetic variation in P acquisition under low P conditions (Lynch, 2007).

To date, there have been many studies on root hairs (Vandamme *et al*., 2013; Wei *et al*., 2016), root exudates (Vengavasi *et al*., 2017; Shujie and Yunfa, 2011), in breeding P efficient soybean germplasm, but less attention on AMF. In soybean, AM colonization enhances the overall water status (Safir *et al*., 1971; Vejsadova *et al*., 1993; Porcel and Ruiz-Lozano, 2004); increases yield and dry matter accumulation (Carling and Brown, 1980; Planchette and Morel, 1996). The symbiosis association between plant roots and fungi is a fair exchangeable resource. They will both received accordingly with what they provided (Kiers *et al*., 2011). Therefore, the enhancement of P uptake in AM- infected plant does not always mean a positive correlation with increasing plant growth (Zhu and Smith, 2001; Zhu *et al.*, 2001). Plant response to AMF colonization varies according to plant species, genetic diversity of AMF, plant growth stages, and environmental factors.

High P fertilized soil or tilled soil is often associated with low AM colonization (Fairchild and Miller, 1990; Khalil *et al*., 1992; Goss and Varennes, 2002). Soybean and wheat cultivars differences in colonization with AM have been documented (Azcon and Ocampo, 1981; Heckman and Angle, 1987). However, other studies of Jakobsen and Nielsen (1983), Koide *et al*. (1988), and Kapulnik and Kushnir (1991), showed no variation in colonization among different genotypes of oat, tomato, wheat, and barley.

Even though mycorrhiza colonization is an important root trait for P acquisition, the mechanisms of root colonization with these fungi is still an open question. Given such conflicting information regarding the effects of AM on crop productivity, genetic variation of AMF colonization especially under field conditions emphasizes the need for more research to understand this aspect of plant nutrition.

In view of the studies summarized above, we carried out two field studies in two continuous years with diverse soybean germplasms to evaluate potential genetic variation in shoot P concentration, biomass production, PUE related traits under normal and low P conditions. The objectives of our research were to: (1) Evaluate genetic variation of shoot P concentration, biomass accumulation, and PUE related traits under both P levels; (2) Evaluate response of soybean genotypes to P levels; (3) Determine mycorrhizal colonization of different soybean genotypes under field conditions and its relation to yield. Collectively, the results of this research will provide valuable information for genetic approaches in breeding of low P tolerance in soybean which is crucial for improving soybean productivity in low input agroecosystem or minimize input requirements in intensive farming.

MATERIALS AND METHODS

Experiments were conducted in Bradford Research Center (BRC), the University of Missouri, Columbia, Missouri, USA, to explore the genetic variation in PUE of diverse soybean genotypes. The soil at BRC is Mexico silt loam (fine, smectitic, mesic Aeric Vertic Epiaqualf) soil. The experiment was laid out as a spit plot design with five replications and two phosphorus levels (with 112 kg Mono Ammonium Phosphate (MAP) ha^{-1} and without P fertilizer application) with 119 soybean genotypes in 2017, and a subset of 20 genotypes in 2018 experiment. The field site was selected because of low soil P availability as indicated by soil test results (Table 4-1) to establish low P and sufficient P treatments. Soil samples were taken two times, once preplant before applying P fertilizer and a second time after harvest. Soil samples were collected with a soil probe to a depth of 16 cm from five locations within each experimental unit. These five subsamples were combined and thoroughly mixed and samples from all experimental units were delivered to the Soil and Plant Testing Lab at the University of Missouri, where samples were air-dried, ground, and passed through a 2 mm sieve. Samples were then analyzed for texture, pH, available P, exchangeable bases (Ca^{2+}, Mg^{2+}, K^{+}) and organic matter, and results of these analyses are summarized in Table 4-1. Low P and sufficient P treatments were replicated and arranged in a randomized complete block with five replications. The experiments were planted on 8th June 2017 and 10th July 2018. Genotypes were planted at a depth of approximately 2.5 cm and a density of 34.5 seeds per m^2 in both years. In 2017, genotypes were planted in 2.43-m long single-row plots with a spacing of 0.76 cm between rows. In 2018, genotypes were planted in four-row plots measuring 3.05 m in length and 3.04 m in width. Pre-emergence herbicide and manual weeding were combined to maintain plots weed free.

The 119 genotypes planted in 2017 are listed in Table 4-2 and included the 41 SoyNAM parents, 24 obsolete cultivars, 2 current commercial cultivars, genotypes that were used as parents for the development of biparental mapping populations, and an assortment of other diverse plant introductions. For the experiment in 2018, 20 entries were selected based on their characteristics determined in 2017 and whether they served as parental lines of mapping populations under development (Table 4-3).

Prior to biomass sampling at the mid pod fill stage (R5.5) plant height of five plants (cm) was measured in each plot. At R5.5, five representative plants in each plot were harvested and dried at 65°C to constant weight, ground to a fine powder using a two grinding steps, one with a Wiley mill to pass a 2 mm screen followed by a second grinding step with a Cyclone mill to pass a 1mm screen. In 2018, shoot samples were collected in one of the two edge rows of each four-row plot. At full maturity (R8), the two middle rows of each plot were harvested to determine grain yield and 100 seed weight. Seeds were ground to fine powder in a Coffee mill. All ground shoot and seed samples were sent for analysis by a Spectro ARCOS ICP-OES at the Agriculture Diagnostics Lab of the University of Arkansas, Fayetteville, AR. Phosphorus contents were calculated by multiplying P concentration with plant shoot dry weight. Physiological P use efficiency (PUE) was defined as the mg of plant dry weight produced per mg of P absorbed by plants, with P uptake defined as total P in the shoot samples (mg plant^{-1}). The P efficiency ratio (relative shoot growth or efficient index, EI) was calculated as the ratio of shoot dry matter production under low (Zero P fertilizer application) to that under adequate P supply (Plus P fertilization) (Ozturk *et al*., 2005).

In 2018, arbuscular mycorrhizal fungi root colonization (AMF-RC) was assessed for five genotypes to examine the relationship between P uptake (P shoot content) and PUE and AMF-RC. Shortly after biomass sampling, each treatment and replicate of the 5 selected genotypes a soil core was collected with a 10-cm diameter auger to a depth of 20 cm. The auger was centered over the tap root and extracted soil samples were washed gently under tap water to collect fine roots. Fine roots samples were cleared by soaking in 10% KOH for 24 hours two times. Then, the KOH was decanted, and root samples were acidified with 1% HCl for 5 minutes. Then HCl was drained, and root samples were stained with Trypan blue in Lacto glycerol 0.05% for 24 hours (Phyllips and Hayman, 1970). The stain was decanted, and excess stain was removed using successive water rinses. Once the rinsate was clear, roots were stored in lactic acid at 4°C until further processing. For observation of AMF-RC, stained root samples were cut into 5 - 10 mm long segments. Thirty root segments were randomly selected, mounted on slides and examined under the microscope at 10x magnification. Fungal colonization was evaluated according to Trouvelot *et al*. (1986) and expressed as mycorrhization percent (M%). The M% was calculated as the proportion of infected roots over total number of root segments.

Weather data were obtained from the weather station located at the Bradford Research Center and are summarized in Table 4-4.

All measurements were subjected to Two-way ANOVA by SAS version 9.4 (SAS Institute, 2009). Analysis of variance (ANOVA) using the PROC MIXED procedure of SAS software was run with replication as random and variety and P levels as fixed effects. Dependent variables included PlHt, BM, P concentration, P content, PUE, seed yield and AMF infection. Using t-test at $P \leq 0.05$ with Tukey's HSD provided by the

ADJUST=Tukey option. PROC CORR in SAS was used to identify correlative relationships between variables, with significant relationships identified at $P \leq 0.05$.

RESULTS

Experiment 1: Screening diverse soybean genotypes for phosphorus use efficiency under field conditions

Analysis of variance for data collected in 2017 from 119 soybean genotypes revealed significant genotype and P treatment effects for all traits, except for plant height which was not influenced by P treatment. Genotype by P treatment interactions were observed for plant height, shoot biomass, and shoot P content but not for shoot P concentration and PUE (Table 4-6).

In both P treatments, shoot biomass and shoot P content were 2 to >3-fold greater in the genotype with the highest accumulation compared to the genotype with the lowest accumulation (Table 4-6). In contrast, the shoot P concentration was less than 2-fold (~1.75 fold) different between the extreme genotypes in both P treatments. Relative shoot biomass produced in the low P vs. the sufficient P treatment ranged between 58.52 and 114.43 % (Table 4-6).

Shoot PUE was higher in low-P than sufficient-P treatments, ranging from 350.22 – 615.32 g BM g P^{-1} and from 321.39 – 562.94 g BM g P^{-1}, respectively (Table 4-5). Genotypes that ranked highest in PUE in both P treatments were Magellan, Bonus, PI548313, and H10. Six genotypes that exhibited the lowest shoot PUE on the average of two P treatments were PI549021A, PI567435B, PI068604-1, and LD01-5907, whereas PI567435B and PI549021A were the lowest in low-P and sufficient-P, respectively.

Traits correlations examined under -P and +P are shown in Tables 4-7 and 4-8, respectively. In general, relationships between studied traits were consistent at both P levels. Biomass production was positively correlated with Plant height and shoot P content. In addition to a positive correlation with shoot biomass, shoot P content also was positively correlated with shoot P concentration. In contrast, PUE was negatively correlated with both shoot P concentration and shoot P content but was not correlated with shoot biomass.

Experiment 2: Confirmation experiment with 20 selected genotypes

Based on Experiment 1, a subset of 20 genotypes were selected to test repeatability of the results obtained in the first year. Analysis of variance results for this experiment mirrored those from Experiment 1 in that significant genotype effects and treatment effects were observed for all traits except for a P treatment effect on plant height (Table 4-9). However, in contrast to Experiment 1 where interaction effects were significant for plant height, shoot biomass, and shoot P content, no significant genotype by P treatment interaction effects were observed for Experiment 2. Similar to Experiment 1, the ranges in shoot biomass and shoot P content were greater than the range in shoot P concentration (Table 4-10). Relative shoot biomass differed significantly among genotypes and ranged from 61.51 to 100.73 %.

Analysis of variance for seed size (100-seed weight), seed P concentration, and seed yield did not find significant genotype by P treatment interactions but revealed significant genotype effects for all traits and significant effects of P treatment on seed P concentration and yield but not 100-seed weight (Table 4-9).

Ranking genotypes from greatest to lowest Relative shoot biomass production identified. Genotypes PI603454, NCROY, PI603171 were the highest ranking in Relative

shoot growth. This ranking is different from the ranking order these genotypes received based on the data collected in the previous season. When ranked according to relative yield production, genotypes PI407848, PI603454, CLOJ095-4-6 were the highest (Figure 4-1). Correlations between plant height, shoot biomass, shoot P concentration, shoot P content, and PUE largely showed the same pattern as for Experiment 1 (Tables 4-11 and 4-12). However, some differences were observed, including, positive correlations between shoot P concentration and shoot biomass and negative correlations between PUE and shoot BM. Relationships of 100-seed weight with shoot P concentration and shoot P content were positive in both P treatments, whereas the correlation was negative with PUE in both cases. Interestingly, seed P concentration was positively correlated with shoot P concentration in the sufficient-P but not the low-P treatment. Yield was not correlated with shoot P concentration and shoot P content in low P conditions, but a positive correlation was detected in the P fertilized treatment. Yield, seed P concentration, and 100-seed weight all were negatively correlated with PUE in the P-fertilized treatment, but only the correlation between 100-seed weight and PUE was significant (negative) in the low-P treatment. Seed yield also was positively associated with plant height in both P treatments.

Categorization of soybean genotypes for P use efficiency:

Genotypes grown in 2018 were categorized into four groups with respect to PUE independently for both P treatments (Figure 4-2) (Fageria, 1993, and Kosar *et al*., 2003). Genotypes Bonus, PI399027, PI561370 were efficient and responsive in adequate P supply while genotypes PI603171, LG056644, 5M20-2-5-2, KS4694, and Macoupin were found efficient and responsive in the low-P treatment. PI399027 was efficient and responsive in the P-fertilized treatment but was inefficient and non-responsive in the low-P treatment. In

contrast, KS4694 was efficient and responsive in low-P treatment but inefficient and non-responsive in the P fertilized treatment. CLOJ095-4-6 and Mustang were efficient and non-responsive, whereas PI407848 and NCROY were inefficient and responsive in both P treatments. PI603454 was efficient and non-responsive in low P but was inefficient and responsive in the sufficient P treatment, and PI424614 exhibited the opposite response.

Arbuscular mycorrhizal association and genotype performance:

Relative AMF root colonization was not influenced by genotype or P treatment in this study (Table 4-13). Colonization of roots was 85% or greater for all genotypes and in both P treatments. Given the high colonization rate and the lack of differences among genotypes and between P treatments, it was not surprising that correlations with any other traits were not significant in either the low-P or the P-sufficient treatment (Tables 4-14 and 4-15).

DISCUSSION

Plant performance under different P treatments

Application of P significantly increased biomass production, shoot P concentration, shoot P content, PUE, seed P concentration, and seed yield of tested genotypes but did not influence plant height or 100 seed weight (Tables 4-6, 4-9). Better plant growth in response to P fertilization when soil P availability is low was expected and consistent with many previous studies with soybean (*e.g.* Bharati *et al*., 1986; Norman, 1978; Kumar *et al*., 2008, Shahid *et al*., 2009; Darwesh *et al*., 2013; Hasan *et al*., 2013; Ochigbo and Bello, 2014; Turuko and Mohammed, 2014; Samandder *et al*., 2018; Feng *et al*., 2021).

As expected, considerable genotypic variation was observed for all measured traits in both P treatments (Tables 4-6 and 4-9). However, the only genotype by P treatment

interactions were observed for Experiment 1 which included 119 genotypes but only for plant height, biomass, and shoot P content and not for PUE (Table 4-6). The limited genotype by P treatment interactions suggest that the specific P treatments imposed in this study may not have been optimal to explore differential responses of soybean genotypes to P availability, and that characterization of soybean genotypes in a single P environment can be useful to make selections for the P-related traits examined in this study. This also is illustrated by the overlap among genotypes that ranked in the top and the bottom 5% for shoot P concentration and shoot PUE under the two P treatments (Table 4-16). It follows that the top and bottom 5% of genotypes for relative shoot biomass production also overlap shoot P concentration or PUE.

Relationship of BM, P concentration, P content and PUE

It also is interesting to note that shoot P concentrations always were strongly negatively correlated with PUE regardless of P treatment and study year (Tables 4-7, 4-8, 4-11, and 4-12). This is in agreement with observations by many others, including Barber *et al*. (1976), Spehar and Souza (1999), and Furlani *et al*. (2002). Given the close relationship between shoot P concentration and shoot PUE, these results suggest that analysis of shoot P concentration could be used as an initial tool to screen and select soybean genotypes for PUE. This would save time and reduce costs and genotypes identified based on P concentration could be studied further in studies that include direct measurements of PUE.

Seed yield and 100 seed weight increased with increasing shoot P concentration and shoot P content in the P-fertilized treatment, but seed yield not related with neither shoot P concentration nor shoot P content in the low-P treatment. The reasons for this are

unclear but could be related to the generally low yields which, at least in part, were the result of the late planting date in 2018. In part, the later planting date in 2018 may also explain some of the genotype by year interactions that were observed when the data of the 20 genotypes that were grown in both years were analyzed (Appendix. Tables 4-1, 4-2, 4-3).

Genotypes included in this study revealed considerable genotypic variation for all measured traits. According to P-efficiency indices and the response to P treatments, soybean genotypes were grouped into 4 groups: Efficient and responsive (ER), Efficient and non-responsive (ENR), Inefficient and responsive (IR), and Inefficient and non-responsive (INR) (Figure 4-3). Genotypes in these categories, particularly those in the efficient categories serve as good candidates for breeding of cultivars that perform better in P limited environments, and for genetic and physiological studies on mechanisms underpinning better performance in low P soils.

Relationship between mycorrhizal colonization, P uptake, and PUE

Arbuscular mycorrhizal fungi (AMF) belong to the phylum *Glomeromycota*. They form association with more than 80% of terrestrial plants (Giovannetti *et al*., 2012; Baum *et al*., 2015; Harikumar, 2017). The fungi use up to 20% of the photosynthetic C for their development (Parniske, 2008). Plant responses to AMF colonization varies depend on environmental conditions and plant and fungal species (Thirkell *et al.*, 2017). Under low P soil, AMF generally improves plant P uptake and increases crop productivity (Smith *et al*., 2003; Bowles *et al*., 2017. However, the impact of AMF colonization can vary, for example, when P availability is sufficient, AMF colonization may suppress direct uptake of P by plant roots (Facelli *et al*., 2010; Smith and Smith, 2011; Smith *et al*., 2011; Thirkell

et al., 2017). The examination of AMF root colonization in the present study revealed very high root colonization percentages (>85%) in all genotypes and treatments and did not reveal any differences (Figure 4-2, Table 4-13). AMF colonization of soybean roots often have been found to be in the range of 45 to 80% but the degree of colonization can vary considerably (Khalil *et al*., 1992; 1994; Cely *et al*., 2016). Given the high colonization rate and the absence of genotype and P treatment effects, it was not surprising that no relationships of AMF colonization with growth, P traits, seed characteristics, or yield were found (Tables 4-14 and 4-15). While no significant effects were observed, the number of genotypes examined was limited to only five and the analysis was only conducted for one location in one year. Thus, the conclusions that can be drawn from this study are limited and more genotypes should be examined in future study as well as under different soil conditions.

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TABLES AND FIGURES

Table 4-1. Soil physical and chemical properties at the field site at the Bradford Research Center in Columbia, Missouri.

Soil test information	Before planting		After harvesting	
	2017	2018	2017	2018
pHs (salt pH)	6.18	6.22	6.38	6.37
Phosphorus (kg ha^{-1})	16.25	15.69	21.70	23.76
Potassium (kg ha^{-1})	233.03	134.61	208.82	217.22
Calcium (kg ha^{-1})	5838.29	5918.21	6229.69	4803.18
Magnesium	681.5	665.2	647	533.5
Organic matter (%)	2.3	2.06	2.09	2.1
Neutralizable acidity (meq/100g)	1.9	1.55	1.3	1.1
Cation exchange capacity (meq/100g)	18.03	17.67	18.13	14.28

Table 4-2. Soybean genotypes used for the 2017 field study, their origin and characteristics.

No.	Entry	Origin	Trait	Quality
1	PI068521_1	PUE 2016	P Concentration	High
2	PI068604_1	PUE 2016	P Concentration	Low
3	PI068732_1	PUE 2016	P Concentration	High
4	PI087620	PUE 2016	P Concentration	High
5	PI091160	PUE 2016	P Concentration	High
6	PI096927	PUE 2016	P Concentration	Low
7	PI153231	PUE 2016	P Concentration	High
8	PI398237	PUE 2016	Root Complexity	High
9	PI398830	PUE 2016	Root Complexity	Low
10	PI399027	Parent of mapping population	Root Complexity	High
11	PI407832A	PUE 2016	Root Complexity	Low
12	PI423890C	PUE 2016	Root Complexity	High
13	PI423927	PUE 2016	Root Complexity	High
14	PI437685D	PUE 2016	P Concentration	High
15	PI437863B	PUE 2016	P Concentration	High
16	PI438335	PUE 2016	P Concentration	High
17	PI458510	PUE 2016	P Concentration	High
18	PI506420	PUE 2016	P Concentration	High
19	PI532463B	PUE 2016	P Concentration	Low
20	PI536635	PUE 2016	P Concentration	High
21	PI548178	PUE 2016	P Concentration	High
22	PI548313	PUE 2016	P Concentration	High
23	PI549021A	PUE 2016	P Concentration	High
24	PI567201D	PUE 2016	Root Complexity	High
25	PI567435B	PUE 2016	P Concentration	High
26	PI567576	PUE 2016	P Concentration	Low
27	PI574477	PUE 2016	Root Complexity	Low
28	PI593258	PUE 2016	P Concentration	High
29	PI594289	PUE 2016	Root Complexity	High
30	PI594410	PUE 2016	Root Complexity	High
31	PI597478B	PUE 2016	P Concentration	High
32	PI603166	PUE 2016	Root Complexity	High
33	PI603454	Parent of mapping population	Root Complexity	Low
34	PI603549	PUE 2016	P Concentration	Low
35	H7	PUE 2016	P-uptake	High
36	H10	PUE 2016	P-uptake	High
37	H17	PUE 2016	P-uptake	High
38	NCROY	Parent of mapping population		
39	PI424405B	Parent of mapping population		

40	PI424614	Parent of mapping population
41	PI407848	Parent of mapping population
42	PI424610	Parent of mapping population
43	PI432359	Parent of mapping population
44	PI442012A	Parent of mapping population
45	PI603171	Parent of mapping population
46	4J105-3-4	NAM line
47	5M20-2-5-2	NAM line
48	CL0J095-4-6	NAM line
49	CL0J173-6-8	NAM line
50	HS6-3976	NAM line
51	IA3023	NAM line, the SoyNAM hub parent
52	LD00-3309	NAM line; Historical line
53	LD01-5907	NAM line
54	LD02-4485	NAM line
55	LD02-9050	NAM line
56	LG00-3372	NAM line
57	LG03-2979	NAM line
58	LG03-3191	NAM line
59	LG04-4717	NAM line
60	LG04-6000	NAM line
61	LG05-4292	NAM line
62	LG05-4317	NAM line
63	LG05-4464	NAM line
64	LG05-4832	NAM line
65	LG90-2550	NAM line
66	LG92-1255	NAM line
67	LG94-1128	NAM line
68	LG94-1906	NAM line
69	LG97-7012	NAM line
70	LG98-1605	NAM line
71	Magellan	NAM line, obsolete commercial line
72	Maverick	NAM line, obsolete commercial line
73	NE3001	NAM line
74	PI398.881	NAM line
75	PI404.188A	NAM line
76	PI427.136	NAM line
77	PI437.169B	NAM line
78	PI507.681B	NAM line
79	PI518.751	NAM line
80	PI561.370	NAM lines
81	PI574.486	NAM line

82	Prohio	NAM line		
83	S06-13640	NAM lines		
84	Skylla	NAM line		
85	TN05-3027	NAM line		
86	U03-100612	NAM line		
87	PI398965	Plant introduction line		
88	PI408255B	Plant introduction line		
89	PI417107	Plant introduction line		
90	Macoupin	Historical line		
91	Scioto	Historical line		
92	Boone	Historical line		
93	Chief	Historical line		
94	Gibson	Historical line		
95	Wabash	Historical line		
96	Perry	Historical line		
97	Clark	Historical line		
98	Clark63	Historical line		
99	Cutler	Historical line		
100	Bonus	Historical line		
101	Union	Historical line		
102	Douglas	Historical line		
103	Sparks	Historical line		
104	Morgan	Historical line		
105	Flyer	Historical line		
106	Corsica	Historical line		
107	KS4694	Historical line		
108	Stressland	Historical line		
109	Mustang	Historical line		
110	Omaha	Historical line		
111	LS93-0375	Historical line		
112	LN97-15076	Historical line		
113	AG3832	Commercial line		
114	P33T72R	Commercial line		
115	PI398426	Plant introduction line	Seed P concentration	Low
116	PI567496	Plant introduction line	Shoot P concentration	Low
117	PI567753C	Plant introduction line	Seed P concentration	High
118	PI538378	Plant introduction line	Seed P concentration	High
119	PI424329	Plant introduction line	Seed P concentration	Low

Table 4-3. Soybean genotypes selected for the 2018 field study and P-related characteristics.

No.	Genotypes	Source	Year of release/Cross	Category		
				Shoot P content	PUE	Root overall
1	5M20-2-5-2	NAM line		Low	High	High
2	Bonus	Historical line	1971	Low	High	High
3	CL0J095-4-6	NAM line		High	Low	Low
4	IA 3023	NAM line		Low	Low	Low
5	KS4694	Historical line	1993	Low		High
6	LD00-3309	NAM line		Low	High	Medium
7	LD02-4485	NAM line				Low
8	LG05-4317	NAM line				High
9	LG05-4464	NAM line			High	Medium
10	Macoupin	Historical line	1930	High	High	Low
11	Mustang	Historical line	1995	High	Low	
12	NCROY	Mapping population for rooting depth	PI407848 x NCROY			
13	PI399027	Mapping population for PUE	PI603171 x PI399027			
14	PI407848	Mapping population for rooting depth	PI407848 x NCROY			
15	PI424614	Mapping population for P content	PI603454 x PI424614			
16	PI561.370	NAM line			High	Low
17	PI603171	Mapping population for PUE	PI603171 x PI399027			
18	PI603454	Mapping population for P content	PI603454 x PI424614			
19	S06-13640	NAM line		High		High
20	Skylla	NAM line		Low	High	High

Table 4-4. Weather data for the 2017 and 2018 growing seasons including temperature and precipitation at the Bradford Research Center.

Month	2017				2018			
	Temperature			Precipitation	Temperature			Precipitation
	Max.	Min.	Average		Max.	Min.	Average	
	°C			mm	°C			mm
May	23.4	11.3	17.3	3.7	27.9	16.2	22.1	2.3
June	28.5	16.5	22.5	2.7	30.6	18.9	24.8	3.8
July	30.6	19.5	25.0	3.8	30.0	18.7	24.4	2.1
August	26.9	15.5	21.2	2.5	30.2	18.5	24.3	2.9
September	27.7	13.5	20.6	0.7	27.0	15.1	21.1	0.7
October	19.9	8.6	14.2	3.2	18.7	6.9	12.8	4.7
November	13.2	1.4	7.3	0.4	6.4	-2.6	1.9	2.1

Table 4-5. Means and ranges for plant height, shoot dry matter, shoot P concentration, shoot P content, and shoot P utilization efficiency (PUE) of 119 soybean genotypes grown under field conditions with and without P fertilization at the Bradford Research Center in 2017.

Traits	P levels	
	Without P applied	With P applied
Plant height (cm)	74.3 (26.6 – 97.4)	74.7 (26.6 – 96.5)
Shoot dry matter (g)	31.3 (15.2 – 54.5)	33.7 (19.6 – 60.7)
Shoot P concentration (g kg^{-1})	2.3 (1.7 – 2.9)	2.5 (1.9 – 3.3)
Shoot P content (g $plant^{-1}$)	0.07 (0.04 – 0.11)	0.08 (0.05 – 0.15)
PUE (g BM g P^{-1})	460.6 (350.2 – 615.3)	418.6 (321.4 – 562.9)

Table 4-6. Means of morphological and physiological plant characteristics of 119 soybean genotypes grown under field conditions at the Bradford Research Center in 2017.

No.	Variables	Plant height		Shoot biomass		Shoot P concentration		Shoot P uptake		PUE		EI
		cm		g		g kg^{-1}		g $plant^{-1}$		g BM gP^{-1}		%
	Genotypes	Without P	With P	Without P	With P	Without P	With P	Without P	With P	Without P	With P	
1	4J105-3-4	79.36	77.76	33.56	35.31	2.1	2.4	0.07	0.09	490	427	95.40
2	5M20-2-5-2	85.28	81.24	26.27	29.09	2.0	2.6	0.05	0.08	522	377	104.80
3	AG3832	75.92	83.40	28.65	30.84	2.2	2.4	0.06	0.07	460	428	93.31
4	Bonus	88.32	92.96	39.01	41.13	1.8	1.9	0.07	0.08	569	563	96.25
5	Boone	85.28	84.64	26.93	31.54	2.2	2.2	0.06	0.07	474	452	86.44
6	Chief	97.40	96.52	32.05	41.36	2.4	2.5	0.08	0.10	424	403	79.38
7	CL0J095-4-6	71.92	72.36	35.38	37.48	2.5	2.8	0.09	0.11	398	373	94.89
8	CL0J173-6-8	77.20	68.12	27.10	28.01	2.6	2.5	0.07	0.07	400	365	98.52
9	Clark	93.68	87.32	27.28	28.01	2.2	2.6	0.06	0.08	475	363	92.42
10	Clark63	86.04	96.48	32.46	33.68	2.2	2.7	0.07	0.09	457	379	99.37
11	Corsica	75.44	77.72	24.07	26.81	2.1	2.4	0.05	0.07	484	472	91.56
12	Cutler	86.28	88.92	42.16	46.51	2.2	2.4	0.09	0.11	457	422	90.87
13	Douglas	96.36	85.20	34.91	43.73	2.2	2.3	0.08	0.10	465	448	83.10
14	Flyer	76.74	77.80	26.13	32.09	2.3	2.5	0.06	0.08	462	403	82.13
15	Gibson	87.76	89.60	27.55	34.79	2.2	2.2	0.06	0.08	460	455	79.50
16	H10	84.56	95.44	33.96	36.74	1.8	2.0	0.06	0.07	573	521	95.83
17	H17	88.84	82.64	31.90	32.14	2.1	2.2	0.07	0.07	474	464	99.97
18	H7	73.80	87.73	25.41	29.20	2.1	2.0	0.05	0.07	472	440	90.40
19	HS6-3976	73.08	79.12	26.07	26.18	2.3	2.3	0.06	0.06	437	451	95.05
20	IA3023	74.32	74.32	27.05	31.18	2.3	2.7	0.06	0.08	457	384	84.86

21	KS4694	87.72	87.72	29.75	29.85	2.4	2.0	0.07	0.06	462	487	100.46
22	LD00-3309	74.70	83.20	19.85	34.08	2.1	2.3	0.04	0.08	517	460	58.52
23	LD01-5907	73.40	76.68	30.76	32.53	2.7	2.7	0.08	0.09	369	379	90.52
24	LD02-4485	84.40	76.20	34.46	35.78	2.4	2.5	0.09	0.09	413	411	98.29
25	LD02-9050	71.24	72.48	20.85	27.73	2.4	2.5	0.05	0.07	429	396	76.27
26	LG00-3372	87.68	89.20	26.06	30.52	2.3	2.1	0.06	0.07	441	470	85.64
27	LG03-2979	79.16	79.52	30.36	32.25	2.3	2.7	0.07	0.09	451	374	94.71
28	LG03-3191	87.96	93.00	33.14	34.88	2.0	2.3	0.07	0.08	514	437	96.06
29	LG04-4717	70.08	67.72	33.21	34.13	2.4	2.6	0.08	0.09	423	386	94.70
30	LG04-6000	77.60	84.16	28.68	30.27	2.5	2.6	0.07	0.08	398	394	95.48
31	LG05-4292	84.12	82.88	30.12	30.42	2.4	2.4	0.07	0.07	418	439	99.84
32	LG05-4317	84.12	87.64	32.46	35.88	2.1	2.3	0.07	0.09	500	459	92.25
33	LG05-4464	76.08	78.88	32.75	35.73	2.1	2.2	0.06	0.08	493	492	94.18
34	LG05-4832	76.24	77.80	23.85	29.08	2.2	2.3	0.05	0.07	471	473	83.60
35	LG90-2550	65.44	58.40	26.24	29.88	2.3	2.6	0.06	0.08	441	398	88.98
36	LG92-1255	69.80	69.40	18.57	30.29	2.3	2.8	0.04	0.09	449	373	61.48
37	LG94-1128	74.44	62.20	29.17	29.97	2.0	2.3	0.06	0.07	490	431	97.55
38	LG94-1906	76.28	74.84	22.36	25.34	2.3	2.6	0.05	0.07	436	392	90.17
39	LG97-7012	69.48	68.96	23.70	26.41	2.5	2.7	0.06	0.07	415	362	91.62
40	LG98-1605	63.80	67.20	29.41	36.09	2.3	2.2	0.07	0.08	427	473	82.10
41	LN97-15076	86.52	86.80	29.21	36.17	2.4	2.4	0.07	0.09	433	432	81.90
42	LS93-0375	75.64	81.16	30.12	34.77	2.2	2.5	0.07	0.09	465	398	87.58
43	Macoupin	91.44	90.20	36.03	42.29	2.2	2.3	0.07	0.10	466	445	84.60
44	Magellan	85.55	89.44	38.16	42.65	1.6	2.0	0.06	0.09	615	531	90.20
45	Maverick	86.56	91.92	31.40	31.36	2.6	2.4	0.08	0.07	409	424	101.20
46	Morgan	91.92	84.30	31.65	31.35	1.9	2.0	0.06	0.06	526	513	101.96
47	Mustang	96.20	90.16	30.17	36.66	2.0	2.5	0.06	0.09	517	407	83.04
48	NCROY	76.32	87.52	28.34	32.60	2.2	2.5	0.06	0.08	448	404	86.75

49	NE3001	54.12	59.68	24.72	27.27	2.2	2.4	0.05	0.06	472	468	90.51
50	Omaha	76.32	81.80	33.38	36.97	2.5	2.8	0.08	0.11	408	357	91.26
51	P33T72R	73.88	70.32	19.34	19.57	2.2	2.5	0.04	0.05	472	408	100.16
52	Perry	86.67	83.16	29.19	29.89	2.2	2.5	0.06	0.08	473	389	99.12
53	PI068521_1	53.05	58.09	38.23	40.31	2.5	2.5	0.10	0.10	398	402	91.12
54	PI068604_1	57.80	65.58	34.24	41.38	2.6	2.8	0.09	0.11	389	349	82.74
55	PI068732_1	88.64	89.88	39.17	43.16	2.1	2.4	0.08	0.10	489	448	87.79
56	PI087620	86.00	87.08	31.98	35.40	2.1	2.3	0.07	0.08	491	437	91.65
57	PI091160	75.52	72.14	27.81	32.45	2.2	2.5	0.06	0.08	507	434	86.59
58	PI096927	55.20	59.08	32.13	37.14	2.2	2.4	0.07	0.09	486	420	86.78
59	PI153231	80.67	75.09	37.82	40.51	2.4	2.7	0.09	0.10	423	419	94.08
60	PI398.881	90.00	82.28	28.79	29.30	2.3	2.6	0.07	0.08	446	404	103.72
61	PI398237	47.80	49.00	49.89	54.15	2.1	2.5	0.10	0.13	489	395	91.00
62	PI398426	82.32	79.64	26.47	29.41	1.9	2.1	0.05	0.06	536	488	91.38
63	PI398830	62.52	55.28	28.70	32.95	2.1	2.7	0.05	0.09	487	382	90.08
64	PI398965	87.56	88.24	27.16	35.97	2.3	2.6	0.06	0.09	438	408	76.92
65	PI399027	66.44	64.44	25.41	33.78	2.3	2.7	0.06	0.09	446	380	75.67
66	PI404.188A	86.16	85.16	30.34	36.30	2.3	2.1	0.07	0.08	461	488	85.37
67	PI407832A	50.92	46.48	21.96	25.15	2.4	2.9	0.05	0.07	425	347	90.93
68	PI407848	57.96	54.84	23.75	26.99	2.7	2.5	0.06	0.07	382	368	88.93
69	PI408255B	56.00	54.52	27.55	27.80	2.2	2.6	0.06	0.07	483	448	97.48
70	PI417107	89.92	76.24	31.73	31.76	2.2	2.3	0.08	0.07	463	449	114.43
71	PI423890C	60.05	62.13	19.98	31.13	2.0	2.3	0.04	0.07	524	440	65.26
72	PI423927	56.60	50.44	30.05	31.35	2.6	2.5	0.08	0.08	398	407	94.62
73	PI424329	55.64	64.84	24.08	25.28	2.2	2.4	0.05	0.06	477	422	98.22
74	PI424405B	95.36	85.32	25.63	29.56	2.1	2.4	0.05	0.07	476	486	87.63
75	PI424610	63.56	59.36	25.62	29.04	2.1	2.5	0.05	0.07	513	404	95.63
76	PI424614	73.36	76.40	25.70	27.54	2.3	2.4	0.06	0.06	438	442	91.11

77	PI427.136	61.88	69.88	22.69	29.08	2.2	2.7	0.05	0.08	486	382	79.86
78	PI432359	87.36	91.32	29.88	37.62	2.2	2.5	0.06	0.09	463	408	81.45
79	PI437.169B	76.36	76.48	26.96	28.30	1.9	2.3	0.05	0.07	564	439	95.23
80	PI437685D	68.80	44.72	34.05	37.46	2.4	2.6	0.08	0.09	434	410	88.22
81	PI437863B	76.84	74.44	24.44	26.63	2.3	2.6	0.06	0.07	461	411	92.37
82	PI438335	63.80	60.07	41.87	.	2.2	2.4	0.10	.	468	402	-
83	PI442012A	59.16	57.16	27.80	29.00	2.1	2.8	0.06	0.08	470	360	98.03
84	PI458510	51.12	57.04	44.44	48.93	2.4	2.6	0.10	0.12	434	420	90.80
85	PI506420	40.72	47.96	37.89	41.58	2.5	2.8	0.09	0.12	408	350	92.07
86	PI507.681B	57.28	70.32	21.48	23.88	2.1	2.5	0.04	0.06	480	421	91.60
87	PI518.751	73.20	69.04	24.32	28.67	2.4	2.5	0.06	0.08	436	363	89.82
88	PI532463B	64.52	66.72	33.98	36.17	2.5	2.7	0.09	0.10	408	364	92.08
89	PI536635	46.64	35.88	24.66	27.98	2.5	3.0	0.06	0.08	412	344	92.32
90	PI538378	70.68	65.00	23.64	26.86	2.1	2.4	0.05	0.06	530	433	89.34
91	PI548178	46.16	45.88	24.06	25.34	2.1	2.5	0.05	0.06	493	400	96.76
92	PI548313	55.98	63.24	32.18	48.48	1.9	2.1	0.06	0.10	610	521	62.68
93	PI549021A	26.63	26.62	18.30	28.06	2.8	3.1	0.05	0.09	393	321	65.94
94	PI561.370	73.48	78.20	35.93	34.49	2.7	2.4	0.10	0.09	378	401	102.69
95	PI567201D	73.49	76.40	34.06	37.91	2.1	2.4	0.07	0.09	475	422	90.52
96	PI567435B	85.84	82.08	33.29	38.88	2.9	2.8	0.10	0.11	350	365	86.01
97	PI567496	55.60	66.30	22.84	30.79	2.6	2.7	0.06	0.08	389	369	72.14
98	PI567576	74.24	76.92	31.64	43.90	2.1	2.4	0.07	0.10	480	428	72.27
99	PI567753C	87.64	75.64	27.85	28.29	2.1	2.3	0.06	0.07	486	424	99.70
100	PI574.486	87.28	86.84	30.88	39.12	2.6	2.7	0.08	0.11	413	372	79.39
101	PI574477	84.32	82.16	30.74	36.51	2.2	2.4	0.07	0.08	478	447	81.81
102	PI593258	73.28	79.72	31.47	41.16	2.3	2.6	0.07	0.11	431	392	77.63
103	PI594289	47.76	49.84	36.73	38.35	2.2	2.2	0.08	0.08	470	466	93.31
104	PI594410	60.93	66.48	34.90	38.94	2.3	2.6	0.07	0.10	466	390	90.07

105	PI597478B	32.10	59.96	25.94	26.30	2.7	2.6	0.07	0.07	370	385	98.81
106	PI603166	70.84	64.72	25.70	27.00	2.0	2.4	0.05	0.07	509	411	97.48
107	PI603171	91.32	89.84	34.35	38.96	2.0	2.3	0.07	0.09	520	442	93.47
108	PI603454	81.72	86.31	34.34	46.47	2.2	2.3	0.07	0.11	469	440	74.20
109	PI603549	87.20	76.20	23.60	27.21	2.4	2.6	0.06	0.07	423	420	92.85
110	Prohio	81.24	84.84	22.51	35.85	2.4	2.6	0.05	0.09	430	392	63.52
111	S06-13640	80.36	79.21	36.07	42.87	1.8	2.2	0.06	0.11	576	408	83.29
112	Scioto	80.46	83.12	34.15	34.22	2.7	2.6	0.09	0.09	383	389	101.17
113	Skylla	62.80	72.44	20.69	29.29	2.5	2.5	0.05	0.07	403	410	73.38
114	Sparks	94.64	93.76	24.21	27.31	2.0	2.1	0.05	0.06	521	473	89.99
115	Stressland	90.24	86.60	26.27	36.58	2.2	2.4	0.06	0.09	459	433	73.35
116	TN05-3027	74.84	80.84	27.47	29.29	2.5	2.5	0.07	0.07	414	406	94.15
117	U03-100612	65.00	56.64	20.00	26.35	2.1	2.5	0.04	0.07	495	391	73.81
118	Union	90.28	91.52	32.66	40.81	2.0	2.2	0.07	0.09	523	509	82.82
119	Wabash	80.17	82.56	29.80	31.36	2.6	2.8	0.08	0.09	417	366	96.79
P value												
Genotype (G)			<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	0.0002
Treatment (T)			0.7104		<0.0001		0.0306		0.0027		0.0078	-
G*T			0.0034		<0.0001		0.8684		0.0140		0.9868	-
CV%			12.37		14.66		18.35		24.42		20.17	17.73
$LSD_{0.05\%}$			8.14		4.22		0.38		0.02		81.37	22.61

Table 4-7. Correlation coefficients for trait relationships of 119 soybean genotypes evaluated at the Bradford Research Center without P fertilizer addition.

Traits	Plant height	Shoot biomass	Shoot P concentration	Shoot P content	PUE
Plant height					
Shoot biomass	**0.144*****				
Shoot P concentration	**-0.141*****	-0.041^{NS}			
Shoot P content	0.024^{NS}	**0.739*****	**0.629*****		
PUE	**0.114****	0.062^{NS}	**-0.969*****	**-0.591*****	

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
NS, not significant.

Table 4-8. Correlation coefficients for trait relationships of 119 soybean genotypes evaluated at the Bradford Research Center with P fertilizer addition.

Traits	Plant height	Shoot biomass	Shoot P concentration	Shoot P content	PUE
Plant height					
Shoot biomass	**0.222*****				
Shoot P concentration	**-0.112****	-0.041^{NS}			
Shoot P content	0.076^{NS}	**0.726*****	**0.643*****		
PUE	**0.100***	0.022^{NS}	**-0.955*****	**-0.624*****	

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
NS, not significant.

Table 4-9. Means and associated analysis of variance results for morphological and physiological characteristics of 20 soybean genotypes grown under field conditions at the Bradford Research Center in 2018. Values are averages of five replications.

Source	Plant height		Biomass		Shoot P concentration		Shoot P uptake		PUE		100 seeds weight		Seed P concentration		Yield		Efficient index
	cm		g		g kg^{-1}		g plant-1		g BM gP^{-1}		g		g kg^{-1}		kg ha^{-1}		%
Genotypes	Without P	With P	Without P	With P	Without P	With P	Without P	With P	Without P	With P	Without P	With P	Without P	With P	Without P	With P	
NCROY	56.80	58.80	10.96	10.96	2.1	2.2	0.02	0.02	487	464	9.07	9.64	5.0	5.2	295.13	471.56	100.00
PI424614	55.60	59.15	15.39	20.91	2.2	2.7	0.03	0.06	465	370	13.78	13.84	7.0	7.2	474.26	499.16	73.60
5M20-2-5-2	54.80	54.10	16.57	23.21	2.2	2.4	0.04	0.06	461	418	14.54	14.33	5.4	5.9	610.14	644.40	71.39
Bonus	52.15	48.80	15.70	21.90	2.0	2.3	0.03	0.05	499	447	13.63	13.65	5.8	6.3	345.40	478.31	71.69
CL0J095-4-6	59.05	63.85	19.29	24.17	2.5	2.7	0.05	0.07	412	379	12.71	13.14	5.6	6.2	550.08	481.19	79.81
IA 3023	58.80	55.20	14.03	22.81	2.4	2.5	0.03	0.06	422	400	13.16	13.63	5.7	6.2	538.79	643.62	61.51
KS4694	55.75	54.10	16.10	17.58	2.3	2.5	0.03	0.04	456	415	13.17	13.89	5.7	6.0	529.35	610.47	91.58
LD00-3309	54.50	58.90	13.14	16.36	2.3	2.4	0.03	0.04	441	421	11.23	11.08	5.5	5.8	757.71	833.10	80.32
LD02-4485	49.45	52.60	14.97	16.05	2.2	2.1	0.03	0.03	454	487	14.22	13.47	5.6	5.7	778.45	754.30	93.27
LG05-4317	63.00	60.95	13.83	17.51	2.1	2.4	0.03	0.04	475	418	12.03	12.23	5.5	5.8	549.30	599.08	78.98
LG05-4464	67.40	64.30	17.86	18.68	2.1	2.5	0.04	0.05	480	409	12.81	12.47	5.6	5.9	583.15	633.32	95.61
Macoupin	51.60	69.25	17.89	25.53	2.2	2.4	0.04	0.06	464	414	13.81	13.96	5.9	6.2	420.19	641.31	70.07
Mustang	47.35	49.65	20.31	20.79	2.4	2.6	0.05	0.05	424	395	13.43	13.04	5.8	6.3	918.49	1048.58	97.69
PI 561.370	44.00	45.00	20.97	22.68	2.5	2.3	0.06	0.05	404	440	15.06	14.17	6.1	5.9	272.81	466.12	92.46
PI399027	52.60	52.25	14.85	21.46	2.4	2.3	0.03	0.05	427	435	13.86	13.96	5.7	6.0	343.16	445.56	69.20
PI407848	53.85	54.40	15.95	17.39	2.1	2.3	0.03	0.04	496	435	8.14	7.46	6.2	6.4	729.69	521.07	91.72
PI603171	52.90	60.20	16.07	16.35	2.0	2.2	0.03	0.04	513	476	9.87	10.05	6.3	6.7	320.34	497.46	98.29
PI603454	61.80	62.00	16.53	16.41	2.3	2.3	0.04	0.04	449	439	14.44	14.23	5.8	5.8	496.47	417.67	100.73
S06-13640	56.65	48.00	15.79	19.25	2.3	2.2	0.04	0.04	444	468	13.32	12.79	5.7	5.8	572.98	653.88	82.03
Skylla	47.35	53.95	17.45	20.28	2.4	2.4	0.04	0.05	424	422	12.23	13.69	5.9	6.2	212.17	496.76	86.05
P value																	
Genotype (G)	<0.0001		0.0029		0.0002		0.0009		0.0004		<0.0001		<0.0001		<0.0001		<0.0001
Treatment (T)	0.1429		<0.0001		<0.0001		<0.0001		<0.0001		0.9238		<0.0001		0.017		-
G * T	0.1366		0.8234		0.1052		0.5082		0.1396		0.1044		0.1048		0.8799		-

Table 4-10. Range of plant height, shoot dry matter, shoot P concentration, shoot P uptake, and P utilization efficiency (PUE), seed P concentration, and seed yield of twenty soybean genotypes grown under field conditions at the Bradford Research Center in 2018.

Parameters	P levels	
	Without P applied	With P applied
Plant height (cm)	54.8 (44.0 – 67.4)	56.3 (45.0 – 69.3)
Shoot dry matter (g)	16.2 (10.9 – 20.9)	19.5 (10.9 – 25.5)
Shoot P concentration (g kg^{-1})	2.2 (1.9 – 2.5)	2.38 (2.1 – 2.7)
Shoot P content (g $plant^{-1}$)	0.04 (0.02 – 0.05)	0.05 (0.02 – 0.07)
PUE (g BM g P^{-1})	454.8 (403.9 – 512.9)	426.8 (370.1 – 486.9)
100 seed weight (g)	12.73 (8.1 – 15.1)	12.7 (7.5 – 14.3)
Seed P concentration (g kg^{-1})	5.77 (4.9 – 7.0)	6.06 (5.2 – 7.2)
Yield (kg ha^{-1})	480.6 (212.2 – 788.5)	582.6 (398.0 – 1089.3)

Table 4-11. Correlation matric of soybean plant growth and P related traits without P fertilizer addition grown under field conditions at the Bradford Research Center in 2018.

Traits	Plant height	Shoot biomass	Shoot P concentration	Shoot P content	PUE	Seed weight	Seed P concentration	Yield
Plant height								
Shoot biomass	**0.2128***							
Shoot P concentration	0.0127^{NS}	**0.3040****						
Shoot P content	0.1838^{NS}	**0.9288*****	**0.6238*****					
PUE	-0.0103^{NS}	**-0.2707****	**-0.9842*****	**-0.5872*****				
Seed weight	**0.3161****	**0.2800****	**0.3616*****	**0.3722*****	**-0.3715*****			
Seed P concentration	-0.0618^{NS}	**0.2385***	0.0753^{NS}	**0.2290***	-0.0321^{NS}	0.1278^{NS}		
Yield	**0.4025*****	0.06623^{NS}	0.0913^{NS}	0.0845^{NS}	-0.0891^{NS}	0.1021^{NS}	0.0176^{NS}	

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
NS, not significant.

Table 4-12. Correlation matrix of soybean plant growth and P related traits with P fertilizer addition grown under field conditions at the Bradford Research Center in 2018.

Traits	Plant height	Shoot biomass	Shoot P concentration	Shoot P content	PUE	Seed weight	Seed P concentration	Yield
Plant height								
Shoot biomass	0.1575^{NS}							
Shoot P concentration	0.0336 ^{NS}	**0.3663*****						
Shoot P content	0.1276 ^{NS}	**0.9016*****	**0.7238*****					
PUE	-0.0239 ^{NS}	**-0.3332****	**-0.9854*****	**-0.6876*****				
Seed weight	0.0189 ^{NS}	**0.3003****	**0.2973**	**0.3515*****	**-0.3055****			
Seed P concentration	-0.0499 ^{NS}	**0.2692****	**0.3566*****	**0.3699*****	**-0.3204****	0.1107 ^{NS}		
Yield	**0.4026*****	**0.2159***	**0.3237****	**0.3063****	**-0.3059****	0.1603^{NS}	0.1344 ^{NS}	

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
NS, not significant.

Table 4-13. Means of morphological and physiological plant characteristics of five soybean genotypes grown under field conditions at the Bradford Research Center in 2018.

Variables	Plant height		Biomass		Shoot P concentration		Shoot P uptake		PUE		100 seeds weight		Seed P concentration		AMF		Yield	
	cm		g plant^{-1}		g kg^{-1}		g plant^{-1}		g BM gP^{-1}		g		g kg^{-1}		%		kg ha^{-1}	
Genotypes	Without P	With P	Without P	With P	Without P	With P	Without P	With P	Without P	With P	Without P	With P	Without P	With P	Without P	With P	Without P	With P
CL0J095-4-6	59.05	63.85	19.29	24.17	2.5	2.7	0.05	0.07	412.21	378.97	12.71	13.14	5.6	6.2	86.00	85.51	529.35	610.47
IA 3023	58.80	55.20	14.03	22.81	2.4	2.5	0.03	0.06	421.47	400.17	13.16	13.63	5.7	6.2	92.06	93.49	757.71	833.10
KS4694	55.75	54.10	16.10	17.58	2.3	2.5	0.03	0.04	455.64	414.67	13.17	13.89	5.7	6.0	94.20	85.67	778.45	754.30
LG05-4317	63.00	60.95	13.83	17.51	2.1	2.4	0.03	0.04	475.43	418.17	12.03	12.23	5.5	5.8	94.38	93.33	420.19	641.31
PI603454	61.80	62.00	16.53	16.41	2.3	2.3	0.04	0.04	449.21	438.52	14.44	14.23	5.8	5.8	93.86	86.93	496.47	417.67
	P value																	
Genotype (G)	0.0001		0.415		0.005		0.060		0.011		<0.0001		0.205		0.378		0.211	
Treatment (T)	0.562		0.183		0.264		0.061		0.252		0.105		0.029		0.255		0.504	
G * T	0.237		0.228		0.662		0.329		0.625		0.651		0.167		0.720		0.850	

Table 4-14. Correlation matrix of five selected genotypes in No P treatment grown under field conditions at the Bradford Research Center in 2018.

Traits	Shoot biomass	Shoot P concentration	Shoot P content	PUE	Seed weight	Seed P concentration	AMF
Shoot P concentration	0.023^{NS}						
Shoot P content	**0.925*****	0.318^{NS}					
PUE	0.034^{NS}	**-0.986*****	-0.275^{NS}				
Seed weight	**0.490***	0.174^{NS}	**0.511***	-0.152^{NS}			
Seed P concentration	0.208^{NS}	**0.620****	0.334^{NS}	**-0.560****	**0.438***		
AMF	0.165^{NS}	-0.268^{NS}	0.093^{NS}	0.257^{NS}	0.244^{NS}	0.076^{NS}	
Yield	**0.449***	0.352^{NS}	**0.492***	-0.266^{NS}	0.392^{NS}	**0.604****	0.033^{NS}

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
NS, not significant.

Table 4-15. Correlation matrix of five selected genotypes with P treatment grown under field conditions at the Bradford Research Center in 2018.

Traits	Shoot biomass	Shoot P concentration	Shoot P content	PUE	Seed weight	Seed P concentration	AMF
Shoot P concentration	**0.666*****						
Shoot P content	**0.958*****	**0.815*****					
PUE	**-0.697*****	**-0.986*****	**-0.840*****				
Seed weight	0.141^{NS}	0.302^{NS}	0.207^{NS}	-0.305^{NS}			
Seed P concentration	**0.532****	**0.795*****	**0.645*****	**-0.763*****	**0.444***		
AMF	-0.168^{NS}	-0.262^{NS}	-0.181^{NS}	0.293^{NS}	-0.213^{NS}	-0.16^{NS}	
Yield	0.250^{NS}	**0.393***	0.341^{NS}	**-0.420***	**0.394***	**0.467***	0.230^{NS}

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
NS, not significant.

Table 4-16. Soybean genotypes that were ranked high and low for shoot biomass, shoot P status, and relative shoot growth.

	Shoot biomass (g)	P concentration (g kg^{-1})	Shoot P content (g $plant^{-1}$)	PUE (g BM gP^{-1})	Relative shoot growth (%)
10 Highest	PI398237 (52.02)	PI549021A (2.95)	PI398237 (0.12)	Magellan (573)	PI417107 (114)
	PI458510 (46.67)	PI567435B (2.85)	PI458510 (0.11)	Bonus (566)	5M20-2-5-2 (105)
	Cutler (44.34)	PI536635 (2.75)	PI567435B (0.11)	PI548313 (566)	PI398.881 (104)
	PI438335 (41.87)	LD01-5907 (2.70)	PI506420 (0.11)	H10 (547)	PI561.370 (103)
	PI068732_1 (41.17)	PI068604_1 (2.70)	CL0J095-4-6 (0.10)	Morgan (519)	Morgan (102)
	Magellan (40.41)	Wabash (2.70)	Cutler (0.10)	Union (516)	Maverick (101)
	PI603454 (40.41)	CL0J095-4-6 (2.65)	PI068521_1 (0.10)	PI398426 (512)	Scioto (101)
	PI548313 (40.33)	Omaha (2.65)	PI068604_1 (0.10)	PI437.169B (501)	KS4694 (100)
	Bonus (40.07)	PI506420 (2.65)	PI438335 (0.10)	Sparks (497)	P33T72R (100)
	PI506420 (39.75)	PI567496 (2.65)	Omaha (0.095)	LG05-4464 (492)	H17 (100)
10 Lowest	PI548178 (24.07)	Union (2.10)	PI423890C (0.06)	Omaha (382)	Skylla (73)
	PI424329 (24.68)	H7 (2.05)	U03-100612 (0.06)	PI506420 (379)	Stressland (73)
	LG92-1255 (24.43)	Sparks (2.00)	NE3001 (0.06)	PI567496 (379)	PI567576 (72)
	LD02-9050 (24.29)	PI398426 (2.00)	PI398426 (0.06)	PI536635 (378)	PI567496 (72)
	LG94-1906 (23.85)	PI548313 (2.00)	PI424329 (0.06)	PI597478B (377)	PI549021A (65)
	PI407832A (23.56)	S06-13640 (2.00)	PI538378 (0.06)	PI407848 (375)	PI423890C (63)
	PI549021A (23.18)	Morgan (1.95)	PI548178 (0.06)	LD01-5907 (374)	Prohio (63)
	U03-100612 (23.18)	H10 (1.90)	Sparks (0.06)	PI068604_1 (369)	PI548313 (62)
	PI507.681B (22.68)	Bonus (1.85)	PI507.681B (0.05)	PI567435B (357)	LG92-1255 (61)
	P33T72R (19.46)	Magellan (1.80)	P33T72R (0.045)	PI549021A (357)	LD00-3309 (58)
LSD	4.22	0.38	0.02	81.37	22.61

Genotypes were ranked based on the numerical values of shoot biomass, shoot P status, and relative biomass production.
Values in parentheses are mean of two P treatment of the respective traits.

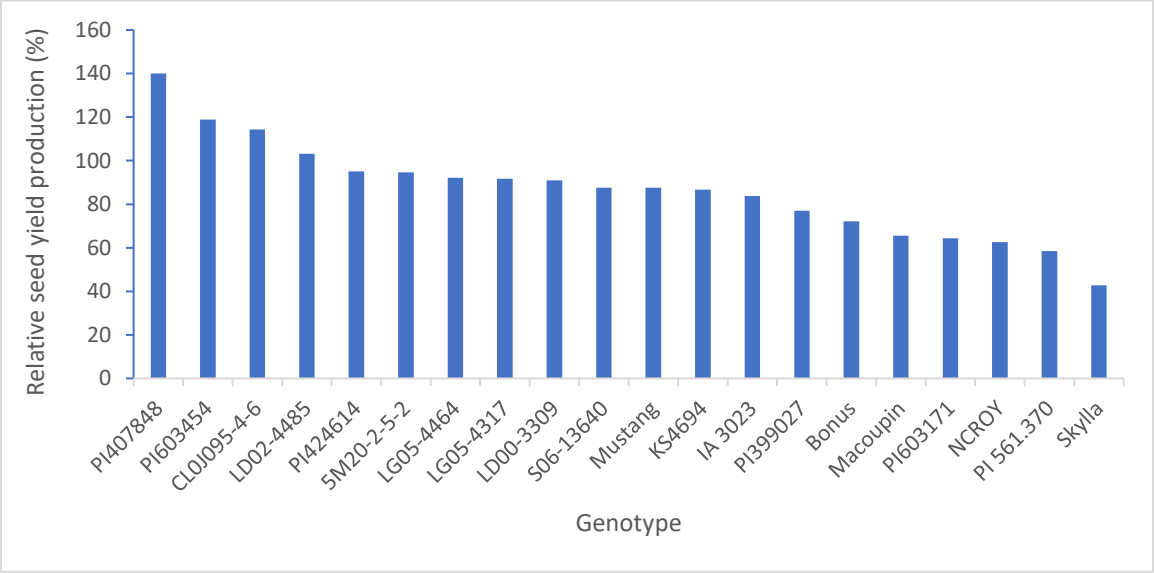


Figure 4-1. Relative seed yield production of soybean genotypes grown in field at the Bradford research center 2018.

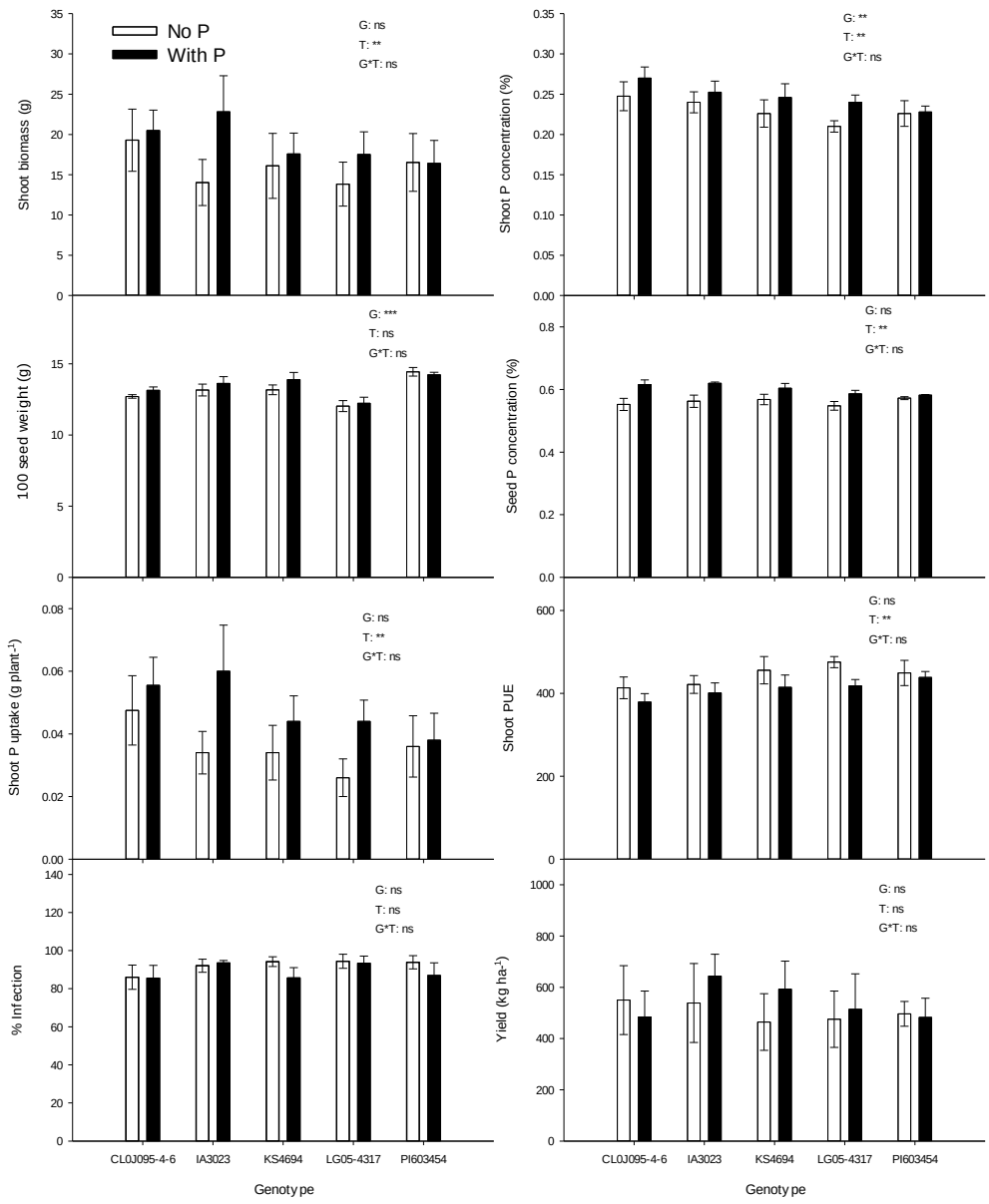


Figure 4-2. Biomass, shoot and seed P concentration, shoot P content, shoot PUE, AMF infection percentage, and seed yield of soybean grown under field condition at the Bradford Research Center in 2018.

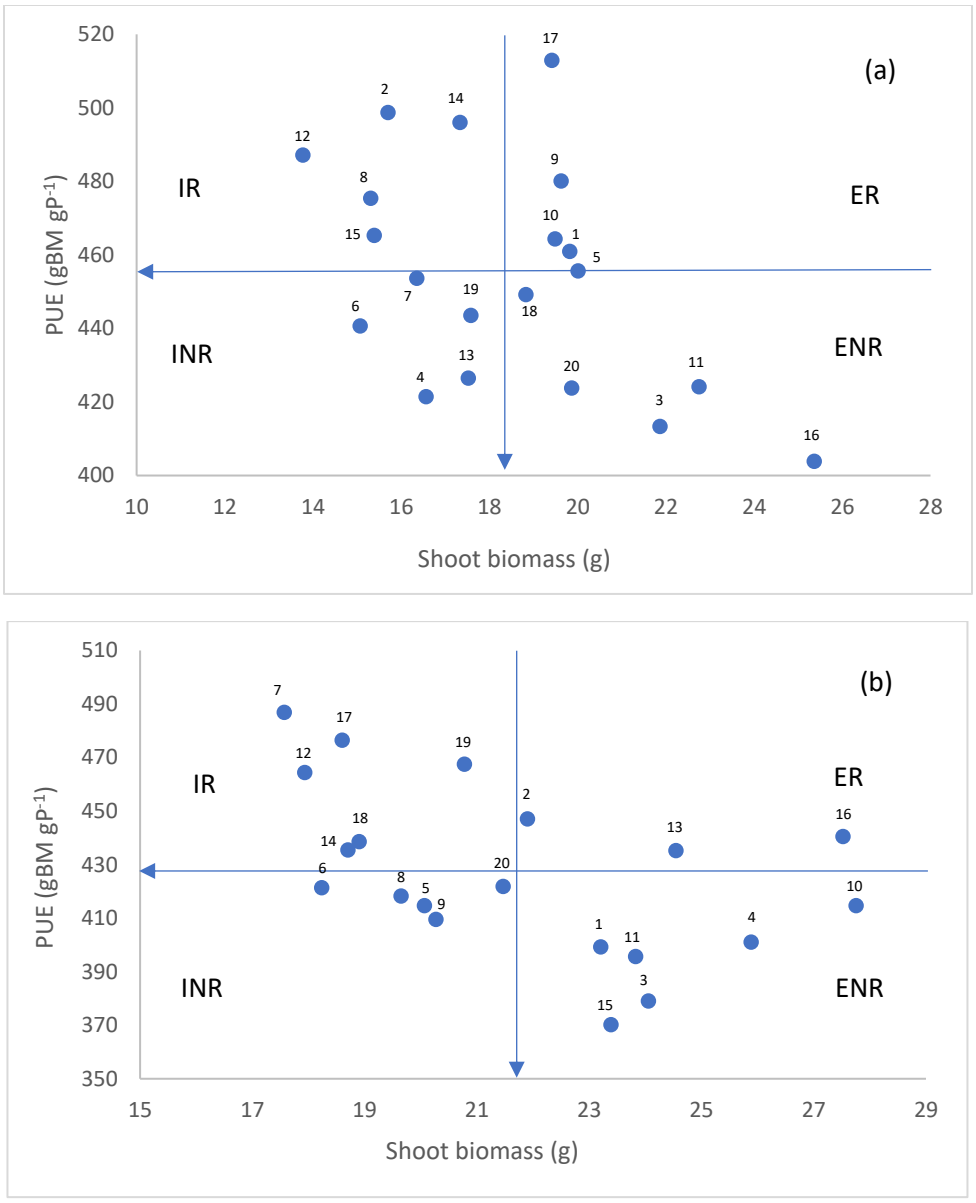


Figure 4-3. Classification of soybean genotypes for P utilization efficiency at a) deficit P and b) adequate P. Data are mean value of five replicates. This categorization divides genotypes into four categories *i.e.* efficient and responsive (ER), in-efficient and responsive (IR), efficient and non-responsive (ENR), and inefficient and non-responsive (INR), whereas the position of each genotype is shown as number from 1 to 20 as specified in Table 4-3.

FUTURE DIRECTIONS

The experiments discussed in this dissertation focused on the exploration of genetic diversity of a broad range of soybean genotypes in terms of P uptake and PUE related phenotypes. The outcomes of these experiments set the stage for a range of follow-up studies but also revealed the limitations of some of the experimental designs and phenotyping efforts employed. For future studies, the genotypes that are the phenotypic tails for the various P- and associated traits are particularly valuable. For instance, considerable variation was found among SoyNAM parental lines, and given that populations already are available, genetic mapping studies could be initiated in short order. Other genotypes identified on the phenotypic tails can be used for the development of biparental mapping populations to pursue a better understanding of the genetic underpinnings of P uptake and PUE in soybean. Importantly, results presented in this dissertation indicate significant genetic variation under both P-sufficient as well as P-limited conditions, thus suggesting potential for genetic improvement for P-uptake, and particularly PUE, for sufficient and limited P availability conditions.

Genotypes contrasting in their P uptake and PUE also are valuable for comparative physiological studies that are aimed at exploring the mechanistic differences resulting in their contrasting responses. It would be particularly advantageous to utilize parental lines of mapping populations or genotypes selected for the development of mapping populations for such studies at this would allow for eventual leveraging of the genetic resources for the elucidation of mechanisms at the molecular level. Follow-up physiological studies should go beyond root architecture and AMF colonization assessment presented in this dissertation

and should include a comprehensive assessment of the contrasting genotypes with respect to phenotypes that have been shown to be involved in P uptake and PUE in plants.

Selection of appropriate study systems will be critical to the success of future experiments. For instance, the pot experiment with eight genotypes described in Chapter 3, was only carried out with two P levels, one that was very low which caused extreme deficiency, and one that was sufficient for plant growth. While the P sufficient treatment allowed for the identification of genotypic differences, no genotypic differences were identified in the low-P treatment, suggesting that the P deficiency likely was too severe to allow expression of genotypic differences in the measured traits. Thus, selection of more appropriate P availability levels will be critical, and the use of multiple levels is recommended. Further, AMF colonization in the selected genotypes was very high under both low-P and P-sufficient conditions. This high level of AMF colonization may not be representative for other locations. Therefore, it will be important to explore soybean responses at multiple field sites to draw broader conclusions. Overall, the findings reported in this dissertation set the state for a broad range of follow-up studies to enhance P uptake and PUE of soybean, which is critical to ensure economic viability and sustainability of soybean production as well as to protect the environment.

APPENDICES

APPENDIX 1

Appendix. Table 2-1. Concentrations of 11 mineral nutrients in shoot tissues of 41 soybean nested association mapping population parental lines that were grown under field conditions at Rollins Bottom in 2015.

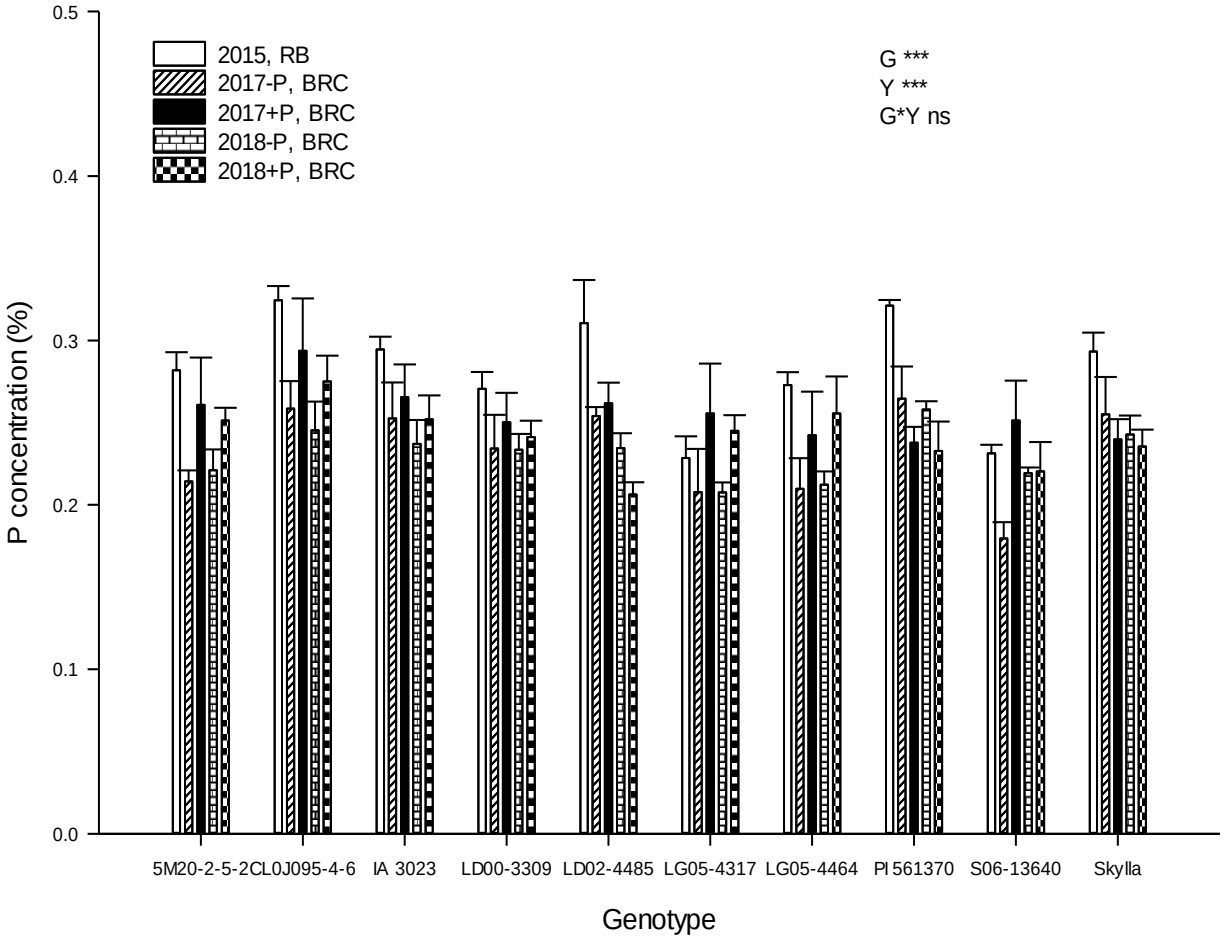
Entry No.	Entry name	P	K	Ca	Mg	S	Na	Fe	Mn	Zn	Cu	Bo
		mg/kg										
1	4J105-3-4	3357.50	16925.00	11382.50	2795.00	1595.00	3.55	56.07	33.59	19.65	7.16	33.06
2	5M20-2-5-2	2817.50	14432.50	11395.00	2867.50	1457.50	1.83	41.51	28.11	16.80	6.32	28.63
3	CL0J095-4-6	3245.00	17722.50	12280.00	2985.00	1642.50	4.76	67.22	32.09	20.49	8.35	31.52
4	CL0J173-6-8	3340.00	17160.00	10705.00	2737.50	1587.50	1.99	48.83	25.76	21.38	7.21	32.69
5	HS6-3976	3105.00	16890.00	13147.50	2857.50	1657.50	2.00	56.52	31.97	20.29	7.10	33.58
6	IA3023	2945.00	16005.00	12215.00	2882.50	1470.00	6.83	58.42	28.47	18.45	7.15	32.78
7	LD00-3309	2705.00	15630.00	11685.00	3087.50	1420.00	1.53	53.46	29.96	17.26	6.77	26.91
8	LD01-5907	3082.50	18377.50	11035.00	3150.00	1655.00	1.46	61.87	33.43	21.08	8.58	28.21
9	LD02-4485	3105.00	18260.00	11797.50	2947.50	1502.50	3.30	54.28	32.06	19.21	7.37	30.11
10	LD02-9050	3132.50	16407.50	10760.00	3140.00	1360.00	3.34	42.94	23.97	17.17	7.38	27.45
11	LG00-3372	2640.00	14755.00	12185.00	2787.50	1537.50	3.05	41.79	27.89	16.13	6.73	29.68
12	LG03-2979	3205.00	16280.00	12025.00	2582.50	1520.00	1.57	62.45	34.51	21.68	7.34	30.50
13	LG03-3191	2777.50	15092.50	10805.00	2780.00	1350.00	1.29	39.17	21.14	15.32	6.93	25.12
14	LG04-4717	2925.00	16892.50	11860.00	2877.50	1460.00	11.87	47.54	26.05	16.27	6.08	31.89
15	LG04-6000	3082.50	16565.00	15720.00	3522.50	1660.00	17.15	53.80	34.67	22.52	7.55	34.06
16	LG05-4292	3137.50	17827.50	11065.00	3250.00	1382.50	2.97	45.78	24.46	17.57	7.28	28.41
17	LG05-4317	2285.00	13847.50	10087.50	2817.50	1272.50	1.67	41.71	22.23	11.64	4.96	26.82

18	LG05-4464	2727.50	16545.00	11020.00	2677.50	1485.00	1.79	40.76	26.25	16.53	5.81	26.63
19	LG05-4832	3065.00	18807.50	10945.00	3307.50	1410.00	3.61	61.38	28.72	18.16	7.65	27.95
20	LG90-2550	2992.50	17977.50	11030.00	2910.00	1552.50	1.24	60.16	30.89	19.67	6.54	31.97
21	LG92-1255	2937.50	15200.00	10770.00	2792.50	1367.50	2.44	48.43	24.52	18.98	6.74	26.72
22	LG94-1128	2752.50	17240.00	11547.50	3360.00	1392.50	3.45	69.06	31.61	19.96	6.21	31.84
23	LG94-1906	2542.50	12855.00	11090.00	2927.50	1270.00	2.06	51.29	24.16	15.65	6.82	26.00
24	LG97-7012	3060.00	16687.50	12792.50	2752.50	1477.50	1.30	58.41	30.65	17.96	8.57	35.22
25	LG98-1605	3005.00	16197.50	11950.00	3170.00	1360.00	0.87	54.84	27.01	17.11	7.63	32.18
26	Magellan	2757.50	16175.00	12210.00	3200.00	1285.00	1.16	50.08	27.28	18.75	6.47	29.45
27	Maverick	2855.00	16097.50	12885.00	2915.00	1535.00	2.60	53.40	33.66	21.51	6.92	29.38
28	NE3001	3145.00	17462.50	11877.50	3337.50	1580.00	2.27	68.71	37.21	20.05	7.09	34.39
29	PI398.881	2862.50	15220.00	11117.50	3055.00	1462.50	7.95	50.10	29.15	19.70	7.24	31.52
30	PI404.188A	2667.50	16947.50	10250.00	3237.50	1215.00	3.47	51.47	27.14	17.43	6.78	29.52
31	PI427.136	2977.50	15312.50	11107.50	2900.00	1487.50	1.92	44.45	27.86	17.00	6.58	30.49
32	PI437.169B	2542.50	14475.00	12007.50	2752.50	1347.50	0.77	49.11	25.52	15.44	5.97	29.31
33	PI507.681B	2627.50	17517.50	11697.50	3542.50	1487.50	0.79	67.96	24.54	13.13	6.26	31.07
34	PI518.751	2912.50	18692.50	10045.00	3107.50	1385.00	3.05	43.02	24.47	14.32	5.66	26.04
35	PI561.370	3212.50	18297.50	9980.00	3075.00	1440.00	1.72	55.53	26.83	19.06	7.18	30.48
36	PI574.486	2782.50	17987.50	10502.50	2960.00	1255.00	2.99	66.27	29.72	17.29	6.45	29.38
37	Prohio	2997.50	17987.50	11322.50	3022.50	1557.50	3.68	45.73	27.47	19.12	7.03	30.59
38	S06-13640	2312.50	11317.50	10407.50	2837.50	1347.50	4.10	36.23	20.30	15.74	5.98	27.84
39	Skylla	2932.50	14987.50	11800.00	3125.00	1287.50	1.29	57.46	28.22	16.32	6.99	28.13
40	TN05-3027	2930.00	14352.50	12160.00	3335.00	1510.00	5.10	48.28	30.20	17.35	7.44	31.77
41	U03-100612	2952.50	16075.00	11802.50	3105.00	1410.00	0.75	60.42	27.17	16.94	6.90	28.89
	Mean	2913.11	16328.90	11523.60	3011.52	1449.70	3.40	52.83	28.31	17.95	6.91	29.96
	LSD	274.50	1908.40	1439.10	336.75	219.12	11.85	16.82	6.15	3.88	0.92	2.71
	CV%	6.73	8.35	8.92	7.99	10.80	184.45	22.74	15.51	15.43	9.55	6.46
	P value	<0.0001	<0.0001	<0.0001	<0.0001	0.0003	0.9791	0.0009	<0.0001	<0.0001	<0.0001	<0.0001

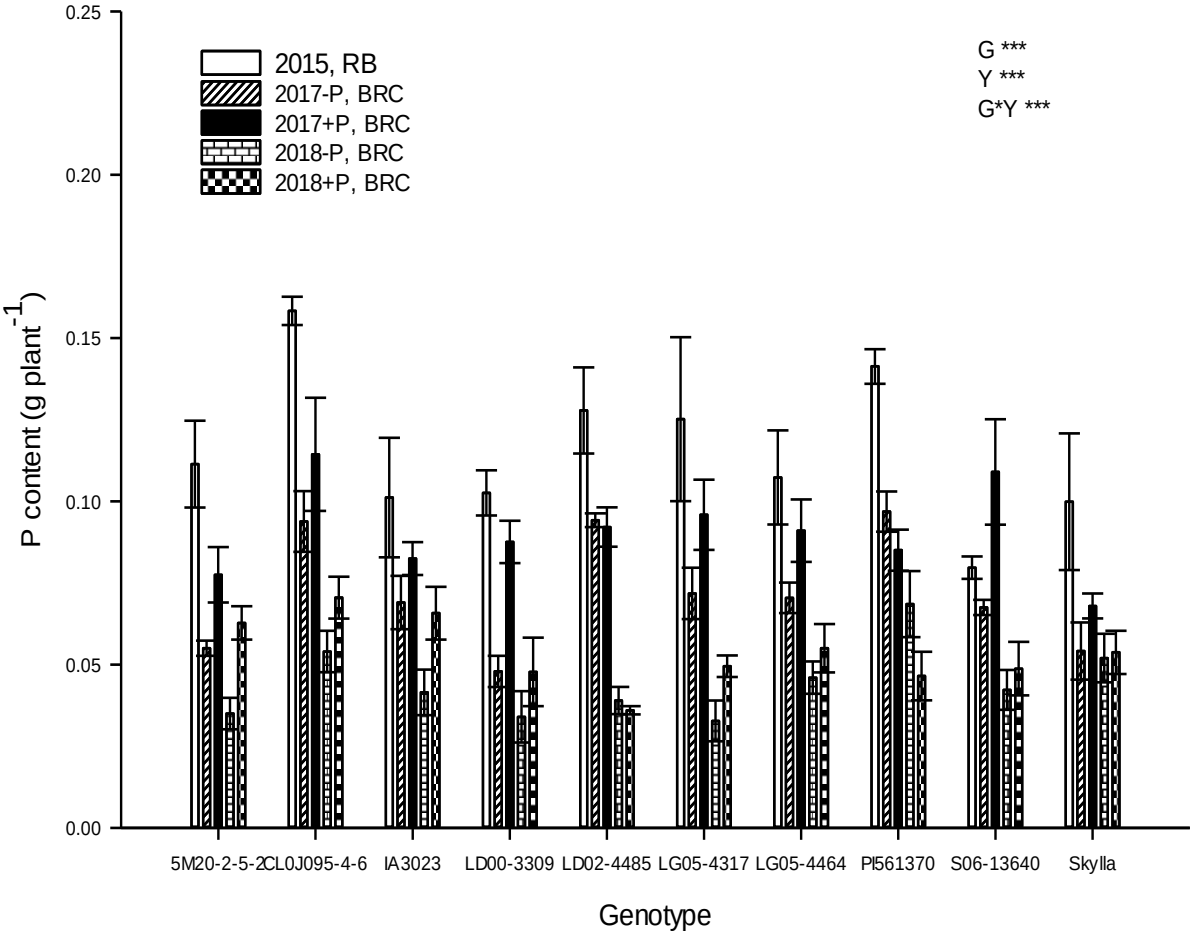
Appendix. Table 2-2. Correlations between shoot biomass (BM) and mineral nutrients concentrations in 41 NAM lines (*Significance is shown as: * P < 0.05; ** P < 0.01 and *** P < 0.001).*

	[P]	[K]	[Ca]	[Mg]	[S]	[Na]	[Fe]	[Mn]	[Zn]	[Cu]	[Bo]
BM	0.11686^{NS}	-0.0191^{NS}	0.07751^{NS}	-0.1024^{NS}	0.17497*	0.02269^{NS}	-0.00668^{NS}	0.2061^{NS}	0.11237^{NS}	0.13448^{NS}	0.04446^{NS}
[P]		0.51178***	0.20786**	-0.0024^{NS}	0.59281***	0.10298^{NS}	0.26438***	0.50433***	0.67959***	0.62783***	0.5005***
[K]			0.04094^{NS}	0.22839**	0.35792***	-0.0812^{NS}	0.25592***	0.33116***	0.30829***	0.15504*	0.25524***
[Ca]				0.30392***	0.42184***	0.18859*	0.28864***	0.62327***	0.41155***	0.24395***	0.60279***
[Mg]					-0.0858^{NS}	0.1336^{NS}	0.2346**	0.10925^{NS}	-0.0709^{NS}	0.04715^{NS}	0.20395**
[S]						0.07834^{NS}	0.29468***	0.70449***	0.72063***	0.45403***	0.53459***
[Na]							-0.00262^{NS}	0.13024^{NS}	0.11157^{NS}	0.06934^{NS}	0.18391*
[Fe]								0.5387***	0.36589***	0.46504***	0.44183***
[Mn]									0.74042***	0.41838***	0.61711***
[Zn]										0.57055***	0.55437***
[Cu]											0.36568***

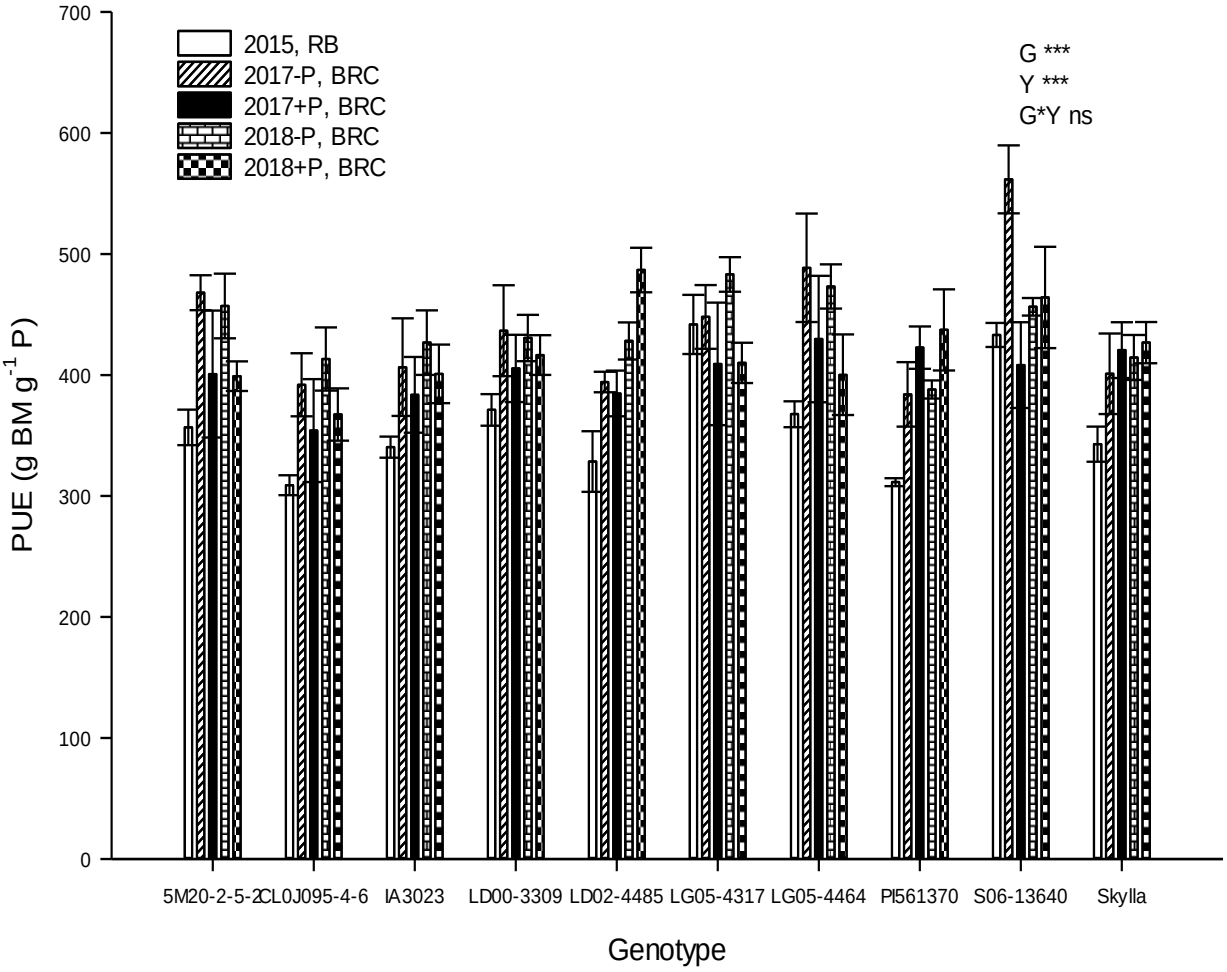
APPENDIX 2



Appendix. Figure 4-1. Shoot P concentration of 10 soybean nested association mapping population parental lines grown under field conditions in three different experiments.



Appendix. Figure 4-2. Shoot P content of 10 soybean nested association mapping population parental lines under different field conditions in three different experiments.



Appendix. Figure 4-3. Shoot P use efficiency of 10 soybean nested association mapping population parental lines grown under field conditions in three different experiments.

Appendix. Table 4-1. Means of plant height, shoot biomass, shoot P concentration, shoot P content, and PUE of 20 soybean genotypes grown under field conditions at the Bradford Research Center in 2017 and 2018.

Sources	Plant height (cm)		Shoot biomass (g)		Shoot P concentration (g kg^{-1})		Shoot P content (g $plant^{-1}$)		PUE (gBM gP^{-1})	
	No P	With P	No P	With P	No P	With P	No P	With P	No P	With P
2017	81.36	82.45	33.12	38.61	2.2	2.5	0.07	0.09	473	428
2018	54.77	56.27	18.96	22.04	2.2	2.4	0.04	0.05	456	430
Means	***68.06***	***69.36***	***26.04***	***30.33***	***2.2***	***2.4***	***0.06***	***0.07***	***464***	***429***
Effect										
Year (Y)	0.0006		<0.0001		0.6234		0.0021		0.7726	
Genotype (G)	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
Treatment (T)	0.0905		0.0068		0.1506		0.0096		0.1763	
G*T	0.1101		0.5023		0.0992		0.0996		0.3650	
Y*G	0.0004		<0.0001		0.0906		<.0001		0.0358	
Y*T	0.7876		0.0559		0.1867		0.0179		0.2724	
Y*G*T	0.5421		0.1006		0.5274		0.7994		0.6138	

Appendix. Table 4-2. Means of plant height, shoot biomass, shoot P concentration, shoot P content, and PUE of 10 soybean nested association mapping population parents grown under field conditions at the Bradford Research Center in 2017 and 2018.

Sources	Plant height (cm)		Shoot biomass (g)		Shoot P concentration (g kg^{-1})		Shoot P content (g $plant^{-1}$)		PUE (gBM gP^{-1})	
	No P	With P	No P	With P	No P	With P	No P	With P	No P	With P
2017	79.57	80.57	30.41	37.61	2.3	2.5	0.07	0.09	474	415
2018	54.01	53.89	19.27	22.04	2.3	2.4	0.05	0.05	436	426
Means	***66.79***	***67.23***	***24.84***	***29.83***	***2.3***	***2.5***	***0.06***	***0.07***	***455***	***421***
Effect										
Year (Y)	<0.0001		<0.0001		0.4783		<0.0001		0.3358	
Genotype (G)	<0.0001		<0.0001		0.0002		<0.0001		0.0031	
Treatment (T)	0.6493		<0.0001		0.001		<0.0001		0.0078	
G*T	0.0945		0.0222		0.0164		0.0222		0.078	
Y*G	0.0536		<0.0001		0.2686		<0.0001		0.1811	
Y*T	0.5597		0.0341		0.0479		0.0341		0.0625	
Y*G*T	0.4554		0.5205		0.8333		0.5205		0.8022	

Appendix. Table 4-3. Mean values from field experiments 2017-2018 for each genotype, treatment, and year for plant height, shoot dry weight, shoot P concentration, shoot P content, and PUE. Means followed by a different letter are significantly different determined by Tukey-Kramer HSD. (α=0.05).

Traits	Plant height (cm)	Shoot biomass (g)	Shoot P concentration (g kg^{-1})	Shoot P content (g $plant^{-1}$)	PUE (gBM gP^{-1})
Genotype					
5M20-2-5	71.79^{DC}	24.10^{FG}	2.25^{CD}	0.054^{GH}	459^{BC}
Bonus	74.15^{BC}	34.18^{A}	1.97^{E}	0.066^{C-F}	523^{A}
CLOJ095-4-6	63.58^{FG}	29.58^{B-E}	2.65^{A}	0.084^{A}	385^{E}
IA3023	64.09^{FG}	25.73^{EFG}	2.50^{AB}	0.064^{C-H}	418^{CDE}
KS4696	75.41^{ABC}	24.69^{FG}	2.25^{CD}	0.055^{FGH}	460^{BC}
LD00-3309	71.09^{CDE}	22.66^{G}	2.29^{BCD}	0.053^{H}	453^{BC}
LD02-4485	67.91^{EDF}	27.27^{DEF}	2.37^{BC}	0.070^{BCD}	428^{CDE}
LG05-4317	71.46^{CDE}	28.38^{C-E}	2.29^{CD}	0.068^{B-E}	447^{CD}
LG05-4464	66.06^{F}	30.68^{A-D}	2.27^{CD}	0.067^{C-F}	460^{AB}
Macoupin	78.16^{AB}	34.08^{A}	2.30^{BCD}	0.080^{AB}	445^{CD}
Mustang	80.10^{A}	31.36^{A-D}	2.35^{BC}	0.070^{BCD}	451^{BC}
NCROY	73.91^{BC}	26.25^{EFG}	2.30^{BCD}	0.057^{E-H}	442^{CD}
PI399027	58.55^{H}	27.35^{DEF}	2.43^{BC}	0.069^{B-E}	415^{CDE}
PI407848	50.99^{I}	22.12^{G}	2.35^{BC}	0.054^{GH}	440^{CD}
PI424614	63.76^{FG}	25.88^{EFG}	2.39^{BC}	0.062^{C-H}	425^{CDE}
PI561370	67.81^{EDF}	27.93^{DEF}	2.50^{AB}	0.074^{ABC}	405^{DE}
PI603171	74.64^{BC}	33.66^{AB}	1.99^{E}	0.066^{C-G}	512^{A}
PI603454	73.81^{BC}	30.61^{A-D}	2.31^{BCD}	0.072^{BCD}	440^{CD}
S06-1364	66.84^{EF}	32.70^{ABC}	2.12^{DE}	0.070^{BCD}	497^{AB}
Skylla	60.10^{GH}	24.11^{FG}	2.44^{BC}	0.060^{D-H}	420^{CDE}
Treatment					
No P	68.03^{A}	25.95^{B}	2.24^{B}	0.059^{B}	461^{A}
With P	69.36^{A}	30.33^{A}	2.41^{A}	0.073^{A}	429^{B}
Year					
2017	81.86^{A}	35.76^{A}	2.35^{A}	0.084^{A}	447^{A}
2018	55.52^{B}	20.50^{B}	2.29^{A}	0.048^{B}	443^{A}
Mean	68.69	28.14	2.32	0.066	445

Appendix. Table 4-4. Correlation matrix for phenotypes of 20 diverse soybean genotypes grown under field conditions at the Bradford Research Center in 2017 and 2018.

Traits	Plant height	Shoot biomass	Shoot P concentration	Shoot P content	PUE
Plant height					
Shoot biomass	**0.6778*****				
Shoot P concentration	-0.0458^{NS}	0.1366**			
Shoot P content	**0.5195*****	**0.8705*****	**0.5888*****		
PUE	0.1061^{NS}	-0.0669^{NS}	**-0.9650*****	**-0.5129*****	

VITA

Thi Van Anh Nguyen was born on July 2nd, 1980, in Hanoi, Vietnam. Anh Nguyen got her Bachelor from the National University of Sciences, Vietnam, with a major in Biotechnology, in 2003. Shortly thereafter, she joined Hybrid Rice Research and Development Center (HRR&DC), Vietnam Academy of Agricultural Sciences (VAAS), as a research assistant. While at HRR&DC, 2005, she received a DANIDA (Danish International Development Agency) scholarship for a Master' degree in the University of Agricultural Sciences, Bangalore, Karnataka, India, under the guidance of Prof. Dr. Rame Gowda. She finished her master's program in Seed Sciences and Technology, in 2007. After coming back from India, she continued work for HRR&DC, and then got promoted and worked for Plant Resource Center, VAAS. Now she becomes a senior research scientist, and personnel of Department of Science and International Cooperation, VAAS. In 2012, Anh Nguyen was awarded a Vietnamese Government Scholarship to pursue her Ph.D. degree in the United States. Start Fall 2014, Anh Nguyen began working towards a Ph.D. in Plant, Insect and Microbial Sciences, the University of Missouri in Columbia, Missouri, under the advisor of Prof. Dr. Felix Fritschi. Her research focused on phosphorus uptake and utilization efficiency in soybean. Upon completion of her Ph.D. in December 2021, Anh Nguyen hopes to find a postdoctoral position to continue her goal of a career in plant science research and education in the United States. After which she plans to get back to her home country to continue contribution to the development of agricultural sciences in Vietnam by either teaching or researching.