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Phosphorus acquisition of soybean genotypes contrasting in root traits

Diplomarbeit

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Diplom-Ingenieur

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Declaration of originality

I hereby certify that this work was written entirely by me. I certify that, to the best of my knowledge, information and data derived from other published or unpublished work are fully acknowledged in the text and references are listed in the list of references.

I declare that this thesis has not been submitted for an academic degree at another University or Institution.

Tulln, October 2015 Favretto Tobia

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Contents

1. Introduction

Phosphorus (P) is one of the 17 mineral nutrients essential for plant growth and is found in every living plant cell. Phosphorus is classified as a major and primary nutrient, meaning that it is frequently deficient in agricultural soils and is required by crops in relatively large amounts.

Phosphorus plays a very important role in absorbing and converting the sun's energy into functional plant compounds (Theodorou and Plaxton, 1993). It is also a vital component of DNA, RNA, and phospholipids in biomembranes. Furthermore, P is required for the synthesis and functioning of ATP, which is considered as «energy currency» in plant metabolism.

Some specific growth factors that have been associated with P are stimulated root development, increased stalk and stem strength, improved flower formation and seed production, more uniform and earlier crop maturity, better N-fixing capacity of legumes, improvements in crop quality, increased resistance to plant diseases and a general supports development throughout the entire plant life cycle.

In the past, as part of a natural cycle, P in manure and waste was commonly returned to the field to sustain and improve crop production. Today the modern terrestrial P cycle is highly influenced by agriculture and human activities (Oelkers and Valsami-Jones, 2008). Because industrial agriculture moves food around the planet for processing and consumption, it disturbs the natural cycle that returned P to the soil via the decomposition of organic matter. In many areas, P-fertilizers must now be constantly applied to maintain soil fertility.

Inorganic P-fertilizers are produced from phosphate rock, a finite resource formed over millions of years in the earth’s crust. Ninety percent of the world’s mined phosphate rock is used in agriculture and for food production, mostly as phosphate fertilizers. Only four countries, i.e. China, Morocco, United States, and Russia, account for more than two-third of the world phosphate rock production. Studies predict that the global commercial phosphate rocks, an unrenewable reserve, will be impoverished within the next century (Steen, 1998).

With a world population that is predicted to reach 9 billion by 2050 and require 70 percent more food than we produce today, and a growing global middle class that is consuming more meat and milk products, adequate P supply will be fundamental to global food

security (FAO 2006). Unfortunately, most of the applied P is wasted. Only 20% of the P in phosphate rock reaches the food consumed globally. Thirty to 40% is lost during mining and processing; 50% is wasted in the food chain between farm and consumer; and only half of all manure is recycled back into farmland around the world. Most of the wasted P flows into rivers, lakes and oceans from agricultural or manure runoff, causing eutrophication (Sanchez 1976).

On the other hand, low soil P fertility leads to problems particularly in humid tropics and subtropics, where high precipitation and warm climates result in soil acidification and weathering (Hue and Ikawa 2001). Wet tropical soils tend to have low concentrations of labile P due in large part to minerals with high P sorption capacities (Sanchez 1976). Crystalline and non-crystalline oxides of iron (Fe) and aluminum (Al) are considered the main geochemical sinks of phosphate in acidic soils (Parfitt et al. 1975; Hsu1977; Lopez-Hernandez 1977; Schwertmann and Taylor 1977; Parfitt 1978). Thus, P is considered to be the most critical nutrient for the production of legumes in tropical and subtropical areas (Raghothama 1999).

Given that phosphate rock is a finite resource and agriculture is the main user of phosphate rock for the production of inorganic P-fertilizers, there is a need to improve both the recycling and use-efficiency of P in agriculture. Developing P-efficient crop genotypes is a key step towards reducing the P input while maintaining or increasing food production (St.Clair and Lynch 2010, Kochian 2012, Vinod and Heuer 2012, Manschadi et al. 2014). The development of crops with better P efficiency, defined as the capacity to grow and produce relevant yields in soils with low P availability, would considerably improve food security in developing countries, while increasing the sustainability of agriculture in rich nations (Lynch, 2007).

Soybean (*Glycine max*) is an annual legume of the *Fabaceae* family, cultivated worldwide for its fatty and protein-rich edible seeds. Soybean is economically the most important legume in the world and is the most nutritious and most easily digested food of the bean family. It is one of the richest and cheapest sources of protein and is a staple in the diet of people and animals in numerous parts of the world.

Soybean plant was first domesticated in China (1500-100 BC), and from there it spread all over the world. Today, the most important soybean producing countries are the US, Brazil, and Argentina (Kumar Gupta et al. 2015). Thanks to its high nutritional value, soybean is an important source of protein and oil for human and animal (Wang et al. 2010). It contains

about 40% protein, 23% carbohydrates, 20% oil, and is rich in secondary nutrients like tocopherols or isoflavonoids.

Soybean is cultivated in tropical, subtropical and temperate areas, often on soils low in P. Low P availability is often a major limit to soybean growth and production. Phosphorus may be a critical constraint of legumes in low-nutrient soils because there is a physiological demand for P in the atmospheric nitrogen fixation process (Sa and Israel 1995). Studies have shown that root morphology and architecture is one the most important traits for efficient P acquisition in soybean (Wang et al., 2004, 2009; Zhao et al., 2004; Cheng et al., 2008; Liu et al., 2008a).

2 State of art

2.1 Phosphorus in the soil

Plant roots absorb P as $H_2PO_4^-$ and HPO_4^{2-}. The mobility of phosphate ions is very low and ranges from approximately 10^{-12} - 10^{-15} m^2 s^{-1} (Schachtman et al. 1998, Havlin et al. 1999). Phosphorus in the soil is in various chemical forms including inorganic P (Pi) and organic P (Po). Organic P generally accounts for 30% to 65% of the total P in soils (Harrison, 1987). These P forms differ in their role and effect in soils (Hansen et al., 2004; Turner et al., 2007). Organic P is found in plant residues, manure and microbial tissues. The Po can become available to plants through mineralization processes mediated by soil organisms and plant roots in association with phosphatase secretion. These processes are highly influenced by soil moisture, temperature, surface physical-chemical properties, and soil pH and Eh (for redox potential). The remainder is in the inorganic fraction containing 170 mineral forms of P. Inorganic forms of soil P consist of apatite, the original source of all P, complexes of iron (Fe) and aluminum (Al) phosphates, and P absorbed onto clay particles. The solubility and the plant availability of these P compounds as well as organic P are extremely low, and only very small amounts of soil P are in solution at any one time.
In acidic soils (Figure 1), P can be absorbed by Al/Fe oxides and hydroxides, such as gibbsite, hematite, and goethite, forming various complexes (Parfitt, 1989). Clay minerals and Fe/Al oxides have large specific surface areas, which provide large number of adsorption sites. The adsorption of soil P can be augmented with increasing ionic strength. With further reactions, P may be closed in nanopores that frequently occur in Fe/Al oxides, and consequently become unavailable to plants (Arai and Sparks, 2007).
In neutral-to-calcareous soils, P retention is dominated by precipitation reactions (Lindsay et al., 1989), although P can also be adsorbed on the surface of Ca carbonate (Larsen, 1967) and clay minerals (Devau et al., 2010). A soil pH between 5.5 and 7 generally results in the highest P availability (Busman et al. 2002).

Between pH values of 4.0 and 6.0, $H_2PO_4^-$ is the dominant form, while HPO_4^{2-} is typical in alkaline soils (Syers et al. 2008, Marschner and Rengel 2012). At high soil pH, most P is in the form of calcium compounds, decreasing in solubility and availability for plants. Reactions that reduce P availability occur in all ranges of soil pH but can be very pronounced in alkaline soils (pH > 7.3) and in acidic soils (pH < 5.5).

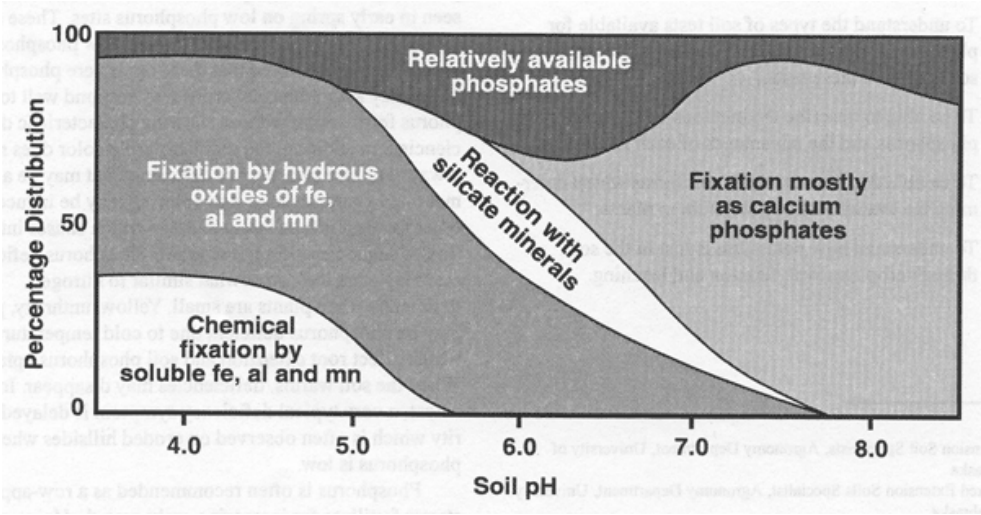

Figure 1: Soil phosphorus compound in relation to soil pH (after Mamo et al. 2013)

Soil P exists in different pools, depending on its availability to plants. The amount of P in the soil solution pool is generally very small. The soil solution P is usually in the orthophosphate form, with the presence of small amounts of organic P. Plants absorbs P only in the orthophosphate form. This is the unique pool that has a measurable mobility.

The active P pool is in the solid phase, which is relatively easily released to the soil solution. As plants adsorb phosphate, the concentration of P in the soil solution decreases and phosphate from the active P pool is thereby released. The capacity of the active P pool to restore the soil solution pool in a soil is a fertility indicator for soil phosphate. Soil particles can act either as a source or a sink of phosphate to the surrounding water depending on conditions.

The fixed P pool of phosphate is represented by very insoluble inorganic phosphate compounds and organic compounds resistant to mineralization. Few slow conversions between the fixed P pool and the active P pool exist in soils. Through fertilization, the amount of fixed P increases. The conversion of available P to fixed P is partly the reason for the low efficiency of P fertilizers. Most of the P fertilizer applied to the soil will not be exploited by the crop in the first season.

2.2 Phosphorus deficiency

Nutrient deficiency in agricultural soils may occur as a result of insufficient quantity and low mobility of nutrients, and/or poor solubility of the abundant chemical forms in the soil. Phosphorus-deficient leaves can show necrotic spots. A major visual symptom is that the plants are dwarfed or stunted. Phosphorus deficient plants develop very slowly compared to other plants growing under similar environmental conditions but without P deficiency. In older leaves under very severe deficiency conditions a brown netted veining of the leaves may develop (Taiz, Zeiger 2010). In some plant species leaves could become bluish green with purple tints. Lower leaves sometimes turn light bronze with purple or brown spots. The shoots are short and thin, upright and spindly (Agrios 2005).

Phosphorus deficiency has been shown to reduce growth of primary roots and enhance length and density of root hairs and lateral roots in many plant species (López-Bucio et al., 2003; Desnos, 2008). Various reports have estimated that carbon expenditure by root systems represents a large fraction of whole-plant carbon budgets under nutrient stress (Eissenstat, 1997). Typical responses of the root system to P starvation include increased root hair length or an increased number of second-order lateral roots (Fageria 2013). Another adaptation mechanism to low-P soil in common bean (*Phaseolus vulgaris*) is shifting biomass allocation to more metabolically efficient root types, such as adventitious roots, which have greater specific root length (SRL, root length per unit biomass, m g^{-1}) and less construction cost than tap and basal roots (Miller et al. 2003). Low P availability reduces lateral root frequency while increasing the extension of the main root axis (Borch et al., 1999).

2.3 Phosphorus efficiency

Variation in nutrient-use efficiency among crop genotypes can be attributed to the efficiency of absorption (acquisition efficiency) and the efficiency with which the absorbed nutrient is utilized to produce the grains (utilization efficiency) (Moll et al. 1982, Manske et al. 2001, Wang et al. 2010). Compared to other essential mineral nutrients, P is the least mobile and least available to plants in many soils. Phosphorus efficiency may be defined as the ability of the plant to produce a certain percentage of its maximum yield at a certain level of soil P. Basically, there are two ways in which different P efficiencies can arise: 1) The efficiency with which P is utilized to produce yield, the amount of P needed in the plant to produce one unit of dry matter (Loneragan and Asher, 1967); 2) The uptake efficiency

of the plant, which is the ability of the root system to acquire P from soil and to accumulate it in the shoots.

This depends on the ability of roots to adsorb P, the active lifetime of roots and the amount of root per unit of shoot. The main strategy for P acquisition used by plants consists of maximal and continued soil exploration through proliferation and extension of all root types with preference for those roots that are metabolically efficient and acquire P avidly (Lynch and Ho, 2005).

2.4 The root system

The plant root system performs various functions, which are essential to growth and development. The plant root system anchors the plant in the soil and provides physical support and at the same time absorbs water, oxygen and nutrients from the soil in mineral solution.

2.4.1 Soybean root system

The root system of many annual dicots, including common bean and soybean, is composed of three main types of axes: a taproot, “basal roots” emerging from the base of the hypocotyl (i.e. the organ between the shoot and the primary root), and adventitious roots emerging from the hypocotyl below the soil surface (Walk et al. 2006).

The tap root differentiates from the radicle and grows vertically downwards. Usually, the tap root penetrates deeply for adequate water uptake. Basal roots generally spread out laterally and are important for nutrient acquisition in upper soil layers. Adventitious roots emerge from other plant organs than the root. In soybean, adventitious roots develop at the hypocotyl. In studies with common bean, basal roots emerged at the base of the hypocotyl, whereas adventitious roots developed from the subterranean portion of the hypocotyl. Thus, adventitious roots are often the shallowest roots of the bean root system. Lateral roots are defined as root branching from any other root. They may appear at all above-mentioned root types (Lynch and Brown 2001, Miller et al. 2003, Lynch 2005, Osmont et al. 2007, Hodge et al. 2009, Zobel and Waisel 2010, Fageria 2013).

2.4.2 Root architecture and morphology

Root architecture is defined as the *in situ* space-filling properties or the spatial distribution of the root system within the rooting volume. For that reason root architecture can be considered as a higher-order organism trait within which traits at the organ and tissue level operate (Lynch & Brown 2001). It is known to play a key role in the capacity of crop plants to acquire soil resources, and hence largely determines their productivity and adaptation to suboptimal soil conditions (Lynch 1995, Fitter 2002, Doussan et al. 2003, Ho et al. 2005, Manschadi et al. 2006, Hodge et al. 2009). Plants are able to respond to P deficiency by changing their root architecture.

Increases in root/shoot ratio, root branching, root elongation, root topsoil foraging, and root hairs are commonly observed in P-deficient plants (Lynch and Brown, 2008; Vance, 2008). Root proliferation is stimulated when plant roots encounter nutrient-rich patches, particularly when the patches are rich in P and/or N (Drew, 1975; Hodge, 2004). A common response to P deficiency is an increase in root-to-shoot dry weight ratio, due to a greater stimulation of root growth at the expense of shoot growth (Mollier and Pellerin, 1999; Hermans et al., 2006).

The movement of P in soils is governed largely by diffusion, so the plant itself contributes to the spatial heterogeneity of phosphorus by depleting it from the rhizosphere (Tinker and Nye, 2000). Another adaptation to P limitation that has been studied mainly in common bean is the exploration of the soil at minimal metabolic cost (Lynch and Brown, 2006 and Lynch, 2007), reducing P requirements of root growth with the induction of aerenchyma in roots (Fan et al., 2003). Cortical cells are replaced with air space and the P released from the breakdown of the cortical tissue could be useful in meeting the P demand for root elongation. Plant root geometry and morphology are important for maximizing P uptake, because root systems that have higher ratios of surface area to volume will more effectively explore a larger volume of soil (Lynch, 1995).

2.4.3 Topsoil foraging

Phosphorus availability usually decreases substantially with depth in agricultural soils because of fertilizer application and cultivation of topsoil and naturally poor mobility of P in the soil profile. The conditions in surface horizons are generally more conducive to P mobilization, because of greater organic matter content, microbial activity, more neutral pH, etc. In agricultural soils, fertilization and cultivation increase P availability in the topsoil, with only very slow movement of phosphorus into the subsoil in most cases. Low P

availability promotes the development of a highly branched root system to the detriment of the primary root, characterized by the stimulated formation and emergence of lateral roots and root hairs (Bates and Lynch, 1996; Williamson et al., 2001; Linkohr et al., 2002; López-Bucio et al., 2002; Pérez-Torres et al., 2008; Péret et al., 2011). Shallower growth of basal roots, increased adventitious rooting, and greater dispersion of lateral roots enable root foraging in the topsoil where P availability is high and contribute to enhanced P efficiency in some plant species (Ge et al. 2000, Lynch & Brown 2001, Miller et al. 2003, Liao et al. 2004a, Ho et al. 2005, Ao et al. 2010, Wang et al. 2010).

The density of roots in the upper soil layers seems to be the most important trait associated with improved acquisition of relatively immobile nutrients such as P. In common bean, topsoil foraging is strongly associated with P acquisition in low P tropical soils (Bonser et al., 1996; Liao et al., 2000; Lynch and Beebe, 1995). Architectural traits associated with promoted topsoil foraging in common bean are shallower basal roots, increased adventitious rooting and greater dispersion of lateral branching from the basal roots (Lynch, 2007; Ramaekers et al., 2010).

There is a positive correlation between the root surface area and the amount of nutrient uptake (Fageria 2013). Therefore, root architecture plays an important role in maximizing P acquisition because root systems with higher surface area are able to explore a given volume of soil more effectively (Lynch, 1995). Root systems with a smaller root diameter and a larger surface area explore the soil more effectively, thereby improving P acquisition (Machado and Furlani 2004). Genotypes with thinner roots showed improved P uptake (Manske et al. 1996).

Lateral roots play also an important role in P acquisition by increasing soil exploration (Zhu et al., 2005) and the absorptive surface of the root system (Pérez-Torres et al., 2008) and P solubilisation (Lynch, 1995, 2007). Phosphorus supply affects the growth and proliferation of lateral roots. Studies have shown that low P involves lateral root growth by reducing primary root elongation and increasing lateral root elongation and density in Arabidopsis (Williamson et al., 2001; Linkohr et al., 2002; Reymond et al., 2006).

2.5. Genetic variability in root characteristics of soybean

In a previous study (Trittinger 2014), thirty-two soybean accessions representing a wide range of origin, date of release, and maturity type were screened for root characteristics. The widely-grown modern Austrian soybean variety ES Mentor was included as the standard genotype.

For root screening, the seedlings of the selected soybean genotypes were grown in a pouch system. The pouch system consisted of (i) a P-free blue blotter paper in A4 size (Anchor Paper Company, USA) with a v-shaped notch (3 cm x 2.5 cm) in the middle of the top edge, (ii) perforated Plexiglas, (iii) clear plastic sheet (Fisherbrand, USA), and (iv) black plastic sheet (Wettlinger Kunststoffe, Austria). Seeds were first surface sterilised for five minutes in a 6% sodium hypochlorite solution and then placed between two sheets of a brown germination paper (Anchor Paper Company, USA) moistened with a 0.5 mM CaSO4 solution. The seeds were positioned 3 cm below the top edge of the paper, having the hilum laterally orientated. The paper was then rolled, fixed with elastic bands, and placed upright into a beaker (5 L volume) that was filled with approximately 500 ml of 0.5 mM CaSO4 solution. The germination rolls were kept for 72 hours at 25°C in a dark germination chamber. The seedlings were then transferred to the pouch system. A seedling with 2-3 cm long primary root was placed in the notch of the blue germination paper with the seed at the back and the primary root on the front of the paper. The perforated Plexiglas was then placed at the back of the blotter paper to stabilize the pouch system. The front side of the blue paper with the root was covered with the clear plastic sheet that was taped to the backside of the Plexiglas. The root-screening unit was then tightly wrapped in a black, lightproof foil except for an opening at the top (about 2.5 x 1 cm) to allow shoot emergence. The pouches were placed at an angle of 15° into custom-made racks standing in rectangular trays (29 x 52 cm) containing 2.2 L of a nutrient solution composed of (mM) 1000 KNO_3, 1000 $Ca(NO_3)_2$, 500 $MgSO_4$, 1000 $NH_4H_2PO_4$, 50 KCl, 25 H_3BO_3, 2 $MnSO_4$, 3 $ZnSO_4$, 0.5 $CuSO_4$, 0.5 $(NH_4)6Mo_7O_{24}$, and 90 Fe-EDTA. The bottom of the pouch system was open to allow the uptake of the nutrient solution. The pouches were arranged in a complete randomized block design with eight replicates per soybean genotype.

The seedlings were grown for 11 days in a controlled-environment growth room with 14h photoperiod, 160 µmoles photons m^{-2} s^{-1} of photosynthetically active radiation, and at a constant temperature of 20 °C. Thereafter, the root systems of soybean seedlings were scanned with a flatbed scanner (CanoScan 5200F) at a resolution of 300 dpi.

The scanned root images were analyzed for morphological root traits using the WinRHIZO Pro software (Regent Instruments Inc., Quebec, Canada). In WinRHIZO Pro, the images were separated in an upper (A) and a lower (B) section (13 x 20 cm each) and root length and diameter were measured in each individual section. This facilitated the identification of genotypes with increased root growth and distribution in the A-Section, which corresponds to the topsoil layer. The growth angle of basal roots (BRGA) was measured at 3 cm distance from the seed relative to a vertical line passing through the stem base using specifically designed computer software. The total number of basal roots (BRNO) was also counted.

The root systems of 14-day old soybean seedlings grown in the pouches exhibited significant genotypic variation in the growth angle (BRGA) and number (BRNO), root length (RL), and root diameter (RD). The cluster analysis of these root traits indicated that the soybean genotypes formed five discrete groups (Fig. 3). The largest groups were Groups 2 and 4 consisting of eight and ten genotypes, respectively. Group 1 and 3 included four genotypes each. Soybean genotypes in Group 1 exhibited a BRGA similar to the overall genotypic mean but their BRNO, RL, and RD were all markedly greater than the genotypic means. Group 2 comprised genotypes expressing relatively shallower BRGA combined with below-average BRNO, RL, and RD. The root characteristics of genotypes in Groups 3 and 4 were similar to the overall average values except for BRNO. Group 5 included genotypes with relatively deep BRGA and low BRNO but with average root length and diameter characteristics.

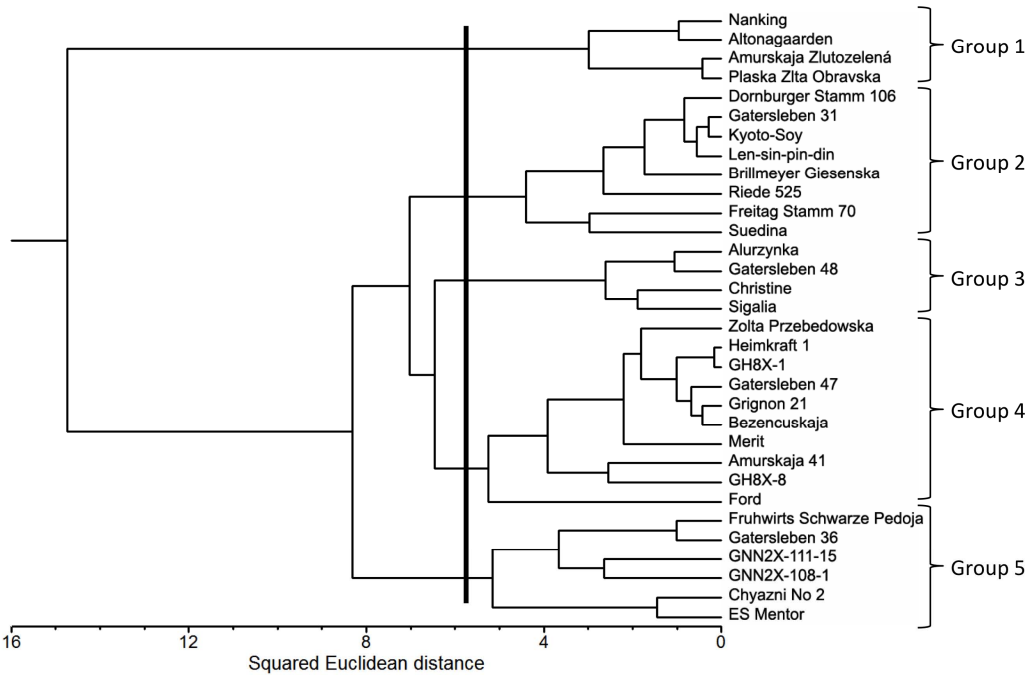

Figure 2: Clustering of 32 soybean genotypes based on their basal root growth angle and number, root length, and root diameter in the pouch system experiment (Trittinger 2014). The horizontal line indicates the cut-off used to form the five groups.

3. Hypotheses and objectives

The cluster analysis presented in Figure 2 suggests significant variation in root traits among the selected set of soybean genotypes. It can be hypothesized that genotypes belonging to the same group would exhibit similar P acquisition levels, while those from different groups would differ markedly in the acquisition of P. Thus, the objective of this study was to quantify the implications of variation in root traits for P acquisition and accumulation at whole-plant level in a subset of contrasting soybean genotypes.

4. Materials and methods

4.1 Plant material and growth substrate

A subset of five soybean genotypes contrasting in root traits was selected from the set screened by Trittinger (2014) to investigate the effects of observed root characteristics in the pouch system on P acquisition at whole-plant level. The selection was based on the cluster analysis of root traits and the seed availability of genotypes. The selected genotypes included ES Mentor, Chyazni No 2, Kyoto-Soy, Riede 525, and Amurskaja Zlutozelená. The standard variety ES Mentor (G1) and Chyazni No 2 (G2) had exhibited very similar root characteristics in the pouch system and were clustered together in Group 5 (Fig. 3). Similarly, Kyoto-Soy (G3) and Riede 525 (G4) were both clustered in Group 2. Amurskaja Zlutozelená (G5) was selected as the representative for Group 1. The reason for selecting two genotypes from the same group (Groups 2 and 5) was to test the hypothesis that genotypes with similar root characteristics, and hence clustering in the same group, would exhibit similarities in root growth and P uptake when they grew in soil-filled pots.

The experimental soil was collected from the top 25 cm surface of a well-fertilized field at the Experimental Farm of the University of Natural Resources and Life Sciences, Vienna, in Groß-Enzersdorf (48°12' N, 16°34' E, 153 m a.s.l.). The soil was classified as Chernozem with pH (CaCl2) 7.2, organic matter content of 2.4%, and a plant-available P content of 81.5 mg kg^{-1}, measured using the calcium-acetate-lactate (CAL)-method (Schüller 1969, ÖNORM L1087 2006). This soil CAL-P level is considered as sufficient for crop growth according to BMLFUW (2006).

The soil was sieved to < 5 mm and mixed with quartz sand (1:1; m:m) to ensure adequate soil aeration and drainage. Given the high soil P content, a pre-experiment was conducted to create a low- and a high-P substrate. In order to do that, Compalox, a granulated activated aluminum-oxide (Al_2O_3) produced by Martinswerk, Bergheim, Germany, was added to the soil. Compalox has a very high affinity for P adsorption and can, therefore, be used to lower plant P-availability in a substrate. The substrate for the pre-experiment was composed of soil and sand in a 4:1 weight ratio. Compalox was added to pots containing 500 g of the soil: sand substrate in 7 different percentages (0%,1%,2%,4%,6%,10%,16%) and each treatment was replicated 3 times. A fleece was placed on the bottom of the pots and then a thick layer of sand as put at the bottom of the pot to facilitate drainage. The

pots were then filled with 500 g of the soil/sand/compalox mixture. The substrate was watered to complete saturation in order to activate the P aluminum-oxide adsorption. The pots were watered to soaking and then allowed to drain overnight. The average gravimetric soil water content was 21, 56% (field capacity). Soil samples were taken 4 days after watering for each pot. The samples were weighed, dried and weighed again to determine the gravimetric soil water content. The soil samples were then analyzed using the CAL methods to determine soil P content in each Compalox treatment.

The CAL (Calcium-Acetate-Lactate) extraction method (Schüller, 1969; Austrian-Standards-Institute, 1993) uses an extraction solution containing 70.6 mM calcium lactate, 50 mM calcium acetate and 0.3 M acetic acid (pH 4.1). All required lab-glass ware (beakers, measuring flasks, test tubes, plastic shaking bottles) were placed in a 5 % nitric acid bath overnight. After 100 ml extraction solution and 5 g of soil air-dried of each pot were shaken for 2 h into 250 ml plastic bottles in an overhead shaker, the suspension was filtrated through pleated filters. The extraction was done with one sample for every pot (3 replicates for 7 Compalox treatments). The filtered solutions were kept in a dark room and were further analyzed within the next 24 hours. Phosphorus concentrations in the filtrate were determined colorimetrically using the molybdate 1 method (blue method 1), where absorbance of the phosphomolybdate complex was measured with a Hitachi U- 200 Spectrophotometer at 660 nm. Calibration standards were prepared using CAL extraction solution in a range between 0.5 and 25 mg/l of P.

Figure XXX shows the relationship between Compalox content of the substrate and CAL-P. The results indicate that substrate-P content decreased markedly with increasing Compalox content. With 10% of Compalox the P content in the substrate was halved.

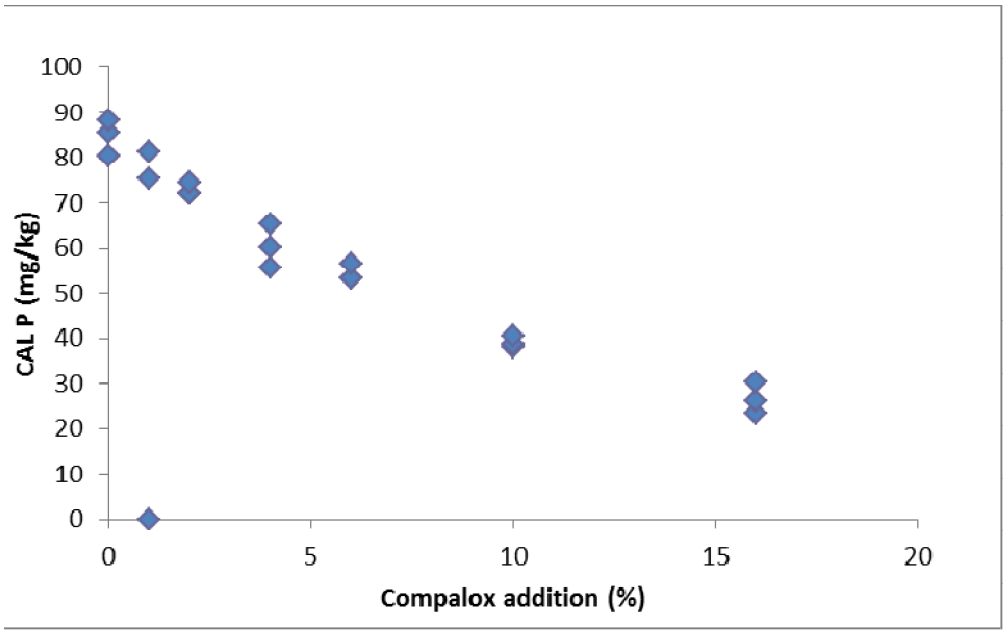

Figure 3: Relationship between % Compalox and plant available phosphorus in a substrate with a 4:1 soil-sand ratio.

As Compalox was not available in sufficient quantity to create a P-level of about 40 mg kg^{-1}, the soil-sand ratio was changed from 4:1 to 1:1. The new substrate was mixed with 4% Compalox and CAL-P was determined after 4 days. The P content was 47.9 mg/kg and it was slightly higher than in the previous experiment (39.0 mg kg-1) but generally with a medium low P availability in the soil mixture.

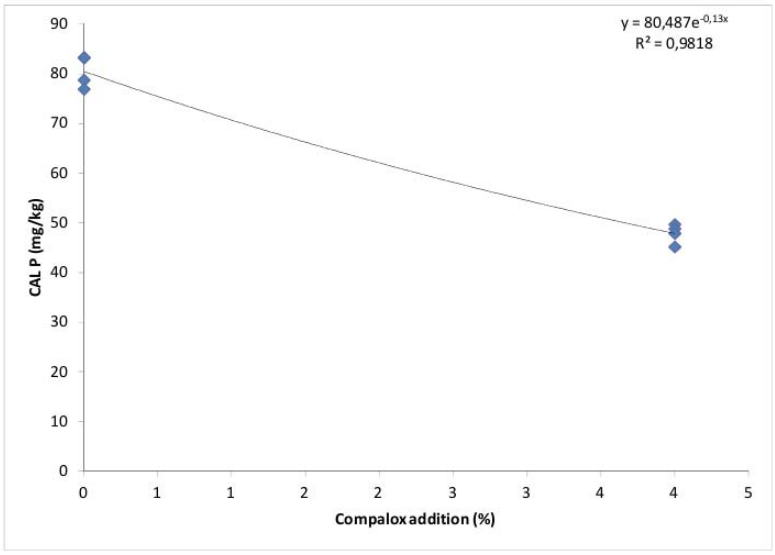

Figure 4: Phosphorus content in a substrate with 4% of compalox and a soil sand ratio of 1:1.

Based on these results, for the low-P treatment (LP), the substrate was amended with 4 % Compalox to lower the CAL-P concentration to 47.9 mg kg^{-1}.

4.2 Experimental setup

Plants were grown in free-draining polyvinylchloride (PVC) columns (25 cm diameter, 40 cm deep). The columns were lined with plastic bags containing 20 kg of air-dried substrate. The substrate column was divided into three layers. The bottom substrate layer (SL-C) was 8 cm, while the middle (SL-B) and upper (SL-A) layers were each 15 cm thick. In both, the low- and the high-P treatments, SL-C and SL-B consisted of the COMPALOX-containing, low-P substrate. In the low-P (LP) columns, SL-A consisted also of the low-P substrate, while COMPALOX-free soil-sand mixture was used for the SL-A in high-P (HP) columns. In this way, low and high P availability was realized in the columns, while the lower substrate layers were always low in available P. Plastic mesh discs (3 mm mesh size) were placed between the substrate layers to facilitate accurate root sampling from the individual layers at plant harvest. The columns were wrapped in an isolation layer (0.5 cm thick), which acted as a thermal insulation layer reducing the diurnal fluctuation in soil temperature.

Three soybean seeds were sown in each column at a depth of 3 cm and the soil was watered with deionized water to field capacity (equivalent to 4100 mL water or 21% gravimetric water content). Following emergence, seedlings were thinned to one per column. The PVC columns were kept well watered (i.e. above 70% field capacity) by regular weighing of a random subset of tubes to determine the quantity of water required to bring the soil water content to field capacity. The PVC tubes were arranged in a complete randomized block design with three replicates per genotype. The experiment was conducted at the Glasshouse of the UFT Campus of the University of Natural Resources and Life Sciences, Vienna, in Tulln, Austria (48°19' N, 16°3' E, 177 m a.s.l.) with a 14 h photoperiod, 22/14 °C day/night temperature, and an average photosynthetic photon flux density of 380 µmol photons m-2 s-1 at pot level.

The fertilizer rates were calculated based on the mineral nutrient contents in the soil and the AGES fertilizers recommendation for soybean in Austria (BMLFUW 2006). The specific salts that were used for the fertilization of the substrate were ammonium nitrate (NH_4)

(NO_3), potassium, chloride (KCl) and ammonium dehydrogenate phosphate ($NH_4H_2PO_4$) (Table 1).

Table 1: Conversion of AGES soybean supply into mg of salts per kg of soil in the pot

	AGES ($kg*ha^{-1}$)	AGES ($mg*kg^{-1}$ soil)	Salt used	Salt MW	AtomW	Salt nutrient%	Salt ($mg*kg^{-1}$ soil)
N	60	28.574	**NH_4NO_3**	80.04	14.01	35.0%	**81.623**
K	93. 4	24.907	**KCl**	74.55	39.1	52.45%	**47.488**
P	28.4	7.573	**NH4H2PO4**	115.1	30.97	26.91%	**28.146**

Depending on the quantity of soil and on the specific P treatment, 3 different soil-salts ratios were arranged. The first type of section was the top soil layer of the tubes under the HP treatment (SL-A); it was composed of 8 kg of substrate, without compalox and with the supply of all the 3 nutritional salts (N/P/K). It was the unique layer where phosphate was added and consequently with a high plant available concentration of P (80 mg/kg). The second type of section was the subsoil of the same treatment (HP).

It was composed of the 2 layers below (SL-B and SL-C), with an overall weight of 12 kg with compalox and with just nitrogen and potassium supply. The LP treatment was characterized by the presence of Compalox in the entire soil mix of the pot (20 kg), fertilized only with N and K in all the 3 layers. The P availability in the subsoil of the HP treatment and in the LP treatment was the same. All fertilizer salts were dissolved in deionized water and adequate volumes of the stock solutions, equivalent to the individual fertilizer rates calculated for each soil layer, were added to small pots containing 500 g of the substrate mix. Following 3 days of drying at room temperature, the soil in each pot was thoroughly mixed with the remaining substrate mix for the soil layer to ensure uniform nutrient distribution and availability. For the high-P treatment (HP), the soil-sand mixture was fertilized with 7.6 mg P kg^{-1}soil (as NH4H2PO4) to counteract the dilution of the soil by the sand. This fertilization maintained the P concentration at 80.5 mg CAL-P kg-1 soil. All columns received 47.5 mg kg-1 KCl (equivalent to 93.4 kg K ha-1) and an equivalent of 120 kg ha-1 N (81.6 mg NH4NO3 kg-1 soil) to suppress nodulation. The fertilizer rates were calculated based on the mineral nutrient contents in the soil and the fertilizers recommendation for soybean in Austria (BMLFUW 2006).

4.3 Data collection

The phenological development of soybean was monitored weekly from emergence to harvest using the BBCH code. At 64 days after sowing (DAS), plant shoots were harvested and dissected into leaves and stems. The dry weight (oven-drying at 65°C for 72 h) of shoot organs was determined for each plant. After plant harvesting, the soil columns were extracted, pulling the plastic bags, and they were cut and separated at the sites of the plastic mesh discs. The soil cylinders were divided into three parts and the top two soil layers (8kg; 8kg) were kept in a cold room to preserve the root tissues until the root washing. After that the roots were separated by the substrate washing it's with water. A metal sieve with 2 mm mesh size was used. The roots collected from each soil layer were stored in a 20% ethanol solution at 4 °C until they were processed.

The total number of basal roots per plant (all roots emerged from the hypocotyl) was determined in the root samples from SL-A. Morphological root traits were measured using a computer-driven scanner and the WinRHIZO software (Regent Instruments Inc., Quebec, Canada). Root dry weight in each soil layer was also determined similar to shoot organs.

For measuring tissue P concentration, plant samples were oven dried (60˚C) and grinded. In each flask with plant material, 10ml of an acid mix composed of HNO3, H2SO4, and HClO4 was added. The day after, the flasks were undergone under 2 specific fusion processes. Subsequently, ortho-phosphate-ions (PO43-) content was analyzed through the Yellow-Method (Ammoniumvanadat-Molybdat-Methode). In acid condition, the H3PO4 ions form the yellow ammoniumphosphorvanadomolybdat-complex, resulting in a yellowish coloration of the solutions.

A spectrophotometer with a wavelength of 436 nm was used to measure the P content of each sample. P standards solutions from 0 ppm to 30 ppm were prepared and measured during the analysis. Varian spectrophotometer was used for that measurement..

4.4 Statistical analysis

Analysis of variance was carried out using the GLM (General Linear Model) procedure of the SAS statistical package (SAS Institute Inc. 1991). Significant differences in the mean values were determined by Tukey test at a significance level of 0.05. A cluster analysis was conducted to identify discrete groups of genotypes with similar root architectural and

morphological characteristics. Clustering was performed in SAS statistical package (SAS Institute Inc. 1991) using Ward's hierarchical approach based on minimum variance linking method with Euclidean distance as the similarity measure (Hartigan 1975). Prior to cluster analysis, the root data were standardized by subtracting the values for each genotype from the overall mean and then dividing by the standard deviation.

5. Results

Plants were harvested 64 days after sowing, at the flowering- early pod stage (69-70 according to the BBCH scale; Munger et al. 1997). There wasn´t significant difference at developmental stage between genotypes.

Root dry matter (RDM)

As shown in Table 2, there were significant differences in root dry matter content between the two treatments. The aluminum oxide Compalox decreased the P availability in the low P treatment, causing a biomass variation within same genotypes. The root dry matter content in the HP treatment exhibited significant higher values in biomass for all genotypes, while plants grown in a low P substrate had a lower root dry matter weight, compared with the same cultivated in a Compalox free pot and with an optimal nutrient content. G4 had the greatest weight for RDM in the HP treatment but not at low P condition where G1 showed the highest value.

Root: shoot ratio (RS-R)

The root shoot ratio expresses the carbon partitioning between root and shoot. Instead of RDM, there were not such relevant variations for RS-R between the two treatments**.** Plants with low available P in the topsoil had a higher root: shoot dry weight ratio (11%). Genotype 1 had the highest RS-R (0.159 ± 0.009) of the cluster groups and on the other hand G2 was the genotype that spent the lowest investments in the development of the underground system.

Specific root length (SRL)

Results for the specific root length (SRL, m root g^{-1} dry mass) showed that genotype 4 and 5 have the highest values for SRL (92.53 ± 2.42 and 87.64 ± 2.16), but without significant variation at P treatment x Gen level. G4 was the greatest genotype for the root length under low P condition and its values were significant higher than the control plant G1 (69.28 ± 1.82). Genotype 3 had the shortest SRL of the group. Although it was not statistically relevant G1 and G4 were the most uniform genotypes for root length, finding 54% of their total root length in the toplayer.

Basal root number (BRNO)

The basal roots were counted after the root washing process. The soil-free roots that emerged from the hypocotyl axis were still fixed at the whole root system of the first layer during counting, without mixing up others different segments with the original basal roots. Basal and adventitious roots were counted together without distinction, because the root diameter differences between these root classes were not easily visible. The basal roots number varied differently in the 2 treatments. On average, genotypes had 25.30 and 22.47 root axes from the hypocotyl in high P and low P treatments. In the low P treatment the BRNO was lower and less vigorous. The variation of basal root number was also significantly influenced by genotype. G4 produced the highest number of BRNO (30.33 ± 1.20), while G5 showed in HP 23.50 ± 0.50 basal roots. At genotype level G5 seemed to have a slightly higher basal root number in the LP. G2 had the less vigorous root system with the lowest number of basal roots in both treatments.

Table 2 Root dry matter (RDM), root shoot weight ratio (RS-R), basal root number (BRNO), and specific root length (SRL) of soybean genotypes grown at high (HP) and low (LP) soil phosphorus levels.

Genotype	RDM (g)			RS-R			SRL (m g^{-1})			BRNO		
	HP	LP	Mean	HP	LP	Mean	HP	LP	Mean	HP	LP	Mean
G1	1.86 a	1.15 a	1.51	0.142	0.175	0.159 a	68.69	69.86	69.28 c	28.67 a b	24.33 a	26.50
G2	0.76 b	0.49 b	0.62	0.086	0.088	0.087 d	86.42	72.43	79.42 b c	19.00 c	18.00 c	18.50
G3	0.68 b	0.49 b	0.58	0.087	0.109	0.098 c d	79.95	71.50	75.72 c	25.00 a b	21.33 b	23.17
G4	2.33 a	1.02 a b	1.67	0.132	0.130	0.131 b	96.01	89.05	92.53 a	30.33 a	23.67 a b	27.00
G5	1.56 a b	0.79 a b	1.17	0.112	0.117	0.115 b c	87.55	87.73	87.64 a b	23.50 b c	25.00 a	24.25
Mean	1.44	0.78		0.112	0.124		83.72	78.11		25.30	22.47	
Sources of variance												
P_level	***			*			***			***		
Gen	***			***			*			***		
P_Level x Gen	**			Ns			ns			**		

***, ** and * indicate significance at the 0.1% (P=0.001), 1% (P=0.01) and 5% (P=0.05) level; ns: nonsignificant; Different letters indicate significant differences at the 5% probability level (Tukey's Test) between genotypes in each column.

Total plant dry matter content (PDM)

As shown in Table 3, the total plant dry matter content was significantly influenced by the different treatments and there were relevant variations in the results between genotypes. At treatment x genotype level there was no significance. The young small pods were weighted together with stem for dry matter content. The HP treatment had an average PDM content of 13.76 g. On the other hand, the low phosphorus condition decreased the whole plant dry weight by 48%, on average respectively. Although it was not significant, G4 had an increase of 22% in PDM compared with the control plant G1. It was the genotype with the greatest PDM (g plant/1) in both treatments. G2 and G3 have the lowest biomass accumulation. G5 had on average similar values in plant dry matter content with the standard cultivar G1.

Phosphorus concentration in leaves (L-Pcon %)

The young small pods were analysed together with stem for P analysis. Results in Table 3 showed significant differences in phosphorus concentration in leaves tissues at treatment level.No significant difference was observed between P level and genotypes. A higher P availability had relevant effects on P concentration in the leaves tissues. G5 has the highest P concentration in leaves (0.344 ± 0.010); G1 and G4 are similar although the control had a slightly higher concentration than G4. Genotype 2 and genotype 3 had the lowest L-P con.%, below the total average.

Phosphorus concentration in stems (S-Pcon%)

G5 also showed, like in the L-Pcon% the highest P concentration in the stem tissue (S-Pcon, 0.409 ± 0.030), while G4 was the genotype with the lowest S-Pcon (0.284 ± 0.023). The control, G2 and G3 had similar values around average.

Phosphorus concentration in roots (R-Pcon%)

Phosphorus concentration in roots was significantly different under P supply level and within genotypes. The P concentrations of the root tissue (R-Pcon in %) were lower than those in the leaves and stem tissues. In the high P treatment, the R-Pcon of G4 (0.174 ± 0.008) was significantly higher than the standard cultivar (G1) and G3 (Table 3). G5 was the genotype with the highest R-Pcon (0.132 ± 0.004) under LP condition.

Table 3 Total plant dry matter (PDM), phosphorus concentration in leaf (L-Pcon), stem (S-Pcon), and roots (R-Pcon) of soybean plants grown at high (HP) and low (LP) soil phosphorus levels.

Genotype	PDM (g)			L-Pcon (%)			S-Pcon (%)			R-Pcon (%)		
	HP	LP	Mean	HP	LP	Mean	HP	LP	Mean	HP	LP	Mean
G1	14.96	7.73	11.35 ab	0.303	0.284	0.294 b	0.381	0.306	0.344 ab	0.125 b	0.112 b	0.118
G2	9.58	6.00	7.79 b	0.235	0.185	0.210 d	0.367	0.357	0.362 a	0.145 ab	0.109 b	0.127
G3	8.73	5.30	7.02 b	0.244	0.240	0.242 c	0.390	0.395	0.393 a	0.137 b	0.107 b	0.122
G4	19.97	9.04	14.50 a	0.296	0.282	0.289 b	0.326	0.242	0.284 b	0.174 a	0.118 b	0.146
G5	15.56	7.51	11.54 ab	0.357	0.332	0.344 a	0.460	0.358	0.409 a	0.151 ab	0.132 a	0.142
Mean	13.76	7.12		0.287	0.265		0.385	0.331		0.146	0.115	
Sources of variance												
P_level	***			***			***			***		
Gen	***			**			**			***		
P_Level x Gen	Ns			Ns			ns			**		

***, ** and * indicate significance at the 0.1% (P=0.001), 1% (P=0.01) and 5% (P=0.05) level; ns: nonsignificant; Different letters indicate significant differences at the 5% probability level (Tukey's Test) between genotypes in each column.

Total root length (RL-T)

The total root length (RL-T, m plant^{-1}) showed in the results significant differences at treatment level, within genotypes and in the interaction between P level and genotype. G4 has the highest total root length among all genotypes in both P supply conditions. Its RL-T (222.35 m plant$^{-1)}$ was much higher than the control genotype G1 (Fig.6). It had precisely 42.9% more total root length compared with the standard cultivar ES Mentor (G1). G4 showed the highest root length under high P. The differences in total root length between genotypes were kept similar at graphical position in both treatments, but with a substantial decrease of length by 51% for the low phosphorus treatment. G4 was also in the low P supply condition the best genotype with the greatest and substantially highest RL-T. Only the differences between G4 and G2 and G4 and G3 were statistically significant. The different phosphorus supply, in the growth substrate, didn't t influence significantly the vertical development of root length in the soil column. However the genotypic effect was relevant. G1 and G4 showed a more homogeneous root length distribution between the different soil layers with 54% of their total root length distributed in the topsoil (SL-A). G5 exhibited a slightly higher portion of the total root length in the toplayer (SL-A), about 62% of its total root system. G2 and G3 showed significantly more root length in the topsoil, about 67%, with a more heterogeneous vertical distribution of RL-T in the soil column.

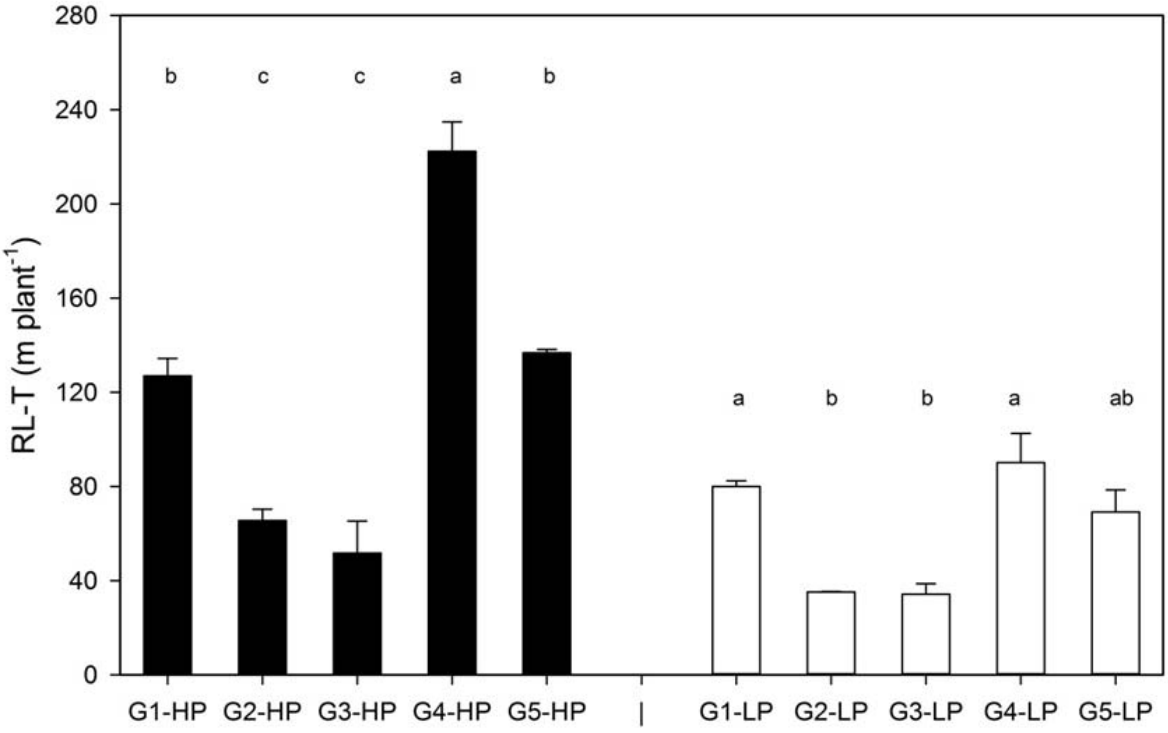

Figure 5: Total root length (RL-T) of soybean genotypes grown in the layered pot experiment. G1, G2, G3, G4, G5 represent soybean genotypes ES Mentor, Chyazni No 2, Kyoto-Soy, Riede 525, and Amurskaja Zlutozelená, respectively. HP and LP denote high and low soil P levels. Values are the means of three replicates ± standard error. Bars within a P treatment with the same letter are not significantly different (p=0.05).

Root diameter

As depicted in Figure 7, the fraction of root length in the topsoil is related with different root diameter classes, from the thinnest RDC1 (<0.2mm) to the thicker class RDC6 (1.0-1.5 mm) under both HP and LP treatments. Just 0.28% in the fraction of total root length of all genotypes was included in the root diameter class 1.5-4.0 mm. The amount of this small portion corresponded to 0.20 m of the total root length of all genotypes, without relevant differences among them. The average root diameter of all plants ranged from 0.371 to 0.480 mm and was significantly influenced by phosphorus supply in the substrate and soybean genotype. In the high P treatment the root diameter was 0.429 mm and in low phosphorus condition the average root diameter grew by 7%. For all genotypes, the majority of the average root length, around 50%, was defined in the root diameter class RDC2 (0.2 – 0.4 mm), for both treatments. The root diameter class RDC6 (1.0 – 1.5 mm) involved just the 1.3% of the total fraction. Genotype 4 showed thinner roots compared

with the others genotypes. The most relevant root diameter class of G4 was RDC1 with 31.9% of the fraction in the HP treatment and 24.5% for the LP. It was significantly thinner in relation to the others genotypes. In the topsoil layer (SL-A), the root diameter of G4 was relevantly lower than that of the others genotypes, under both high (0.375 ± 0.007 mm) and low P treatment (0.413 ± 0.006 mm). In the second layer (SL-B), below the topsoil, there were not significant differences in root diameter compared to the upper layer, at P condition and genotype level. In the second one, G4 was again the genotype with the thinnest root diameter.

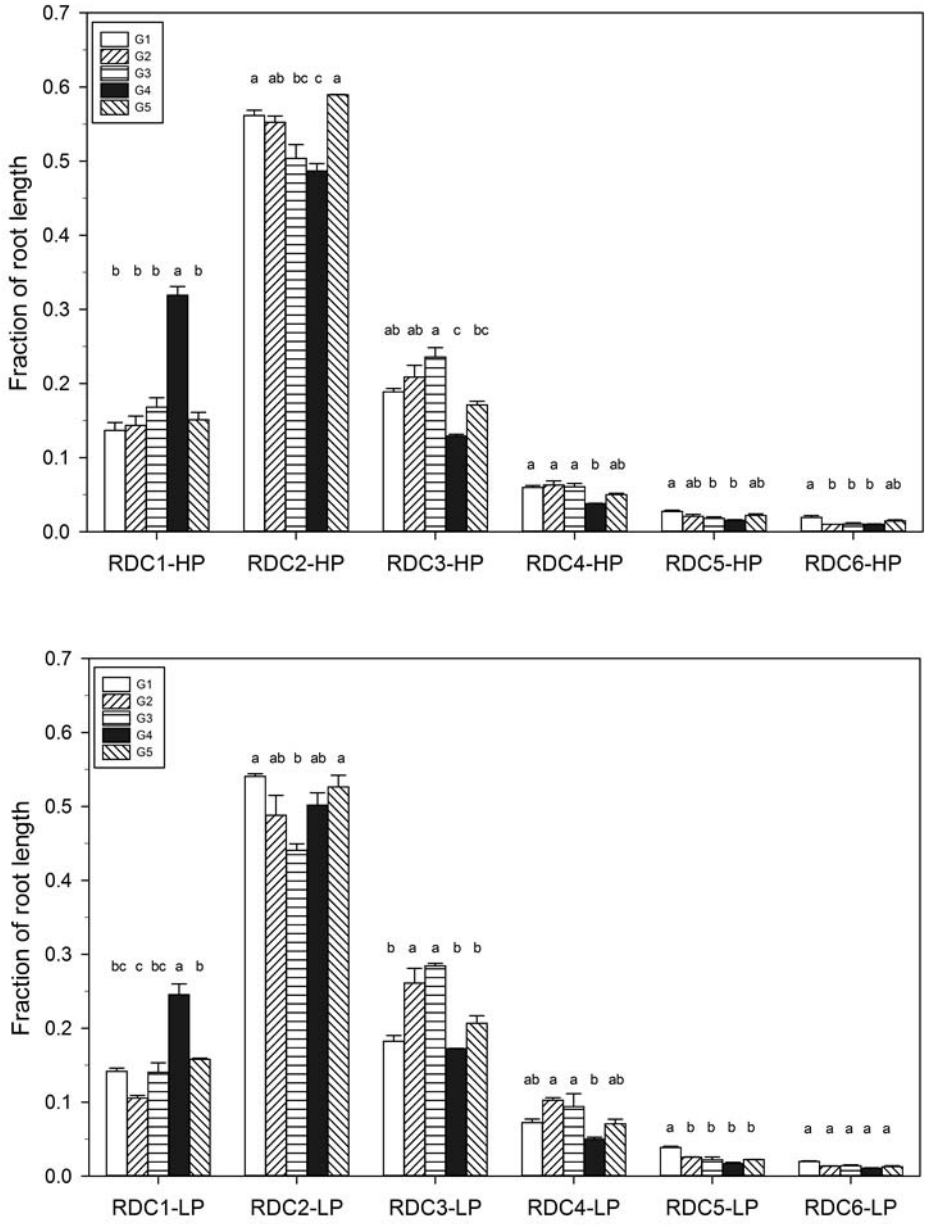

Figure 6: Fraction of root length of soybean plants in individual root diameter classes (RDC). RDC1 to RDC6 indicate root diameters of <0.2, 0.2-0.4, 0.4-0.6, 0.6-0.8, 0.8-1.0, and 1.0-1.5 mm, respectively. G1, G2, G3, G4, G5 represent soybean genotypes ES Mentor, Chyazni No 2, Kyoto-Soy, Riede 525, and Amurskaja Zlutozelená, respectively. HP and LP denote high and low soil P levels. Values are the means of three replicates ± standard error. Bars within a diameter class with the same letter are not significantly different (p=0.05).

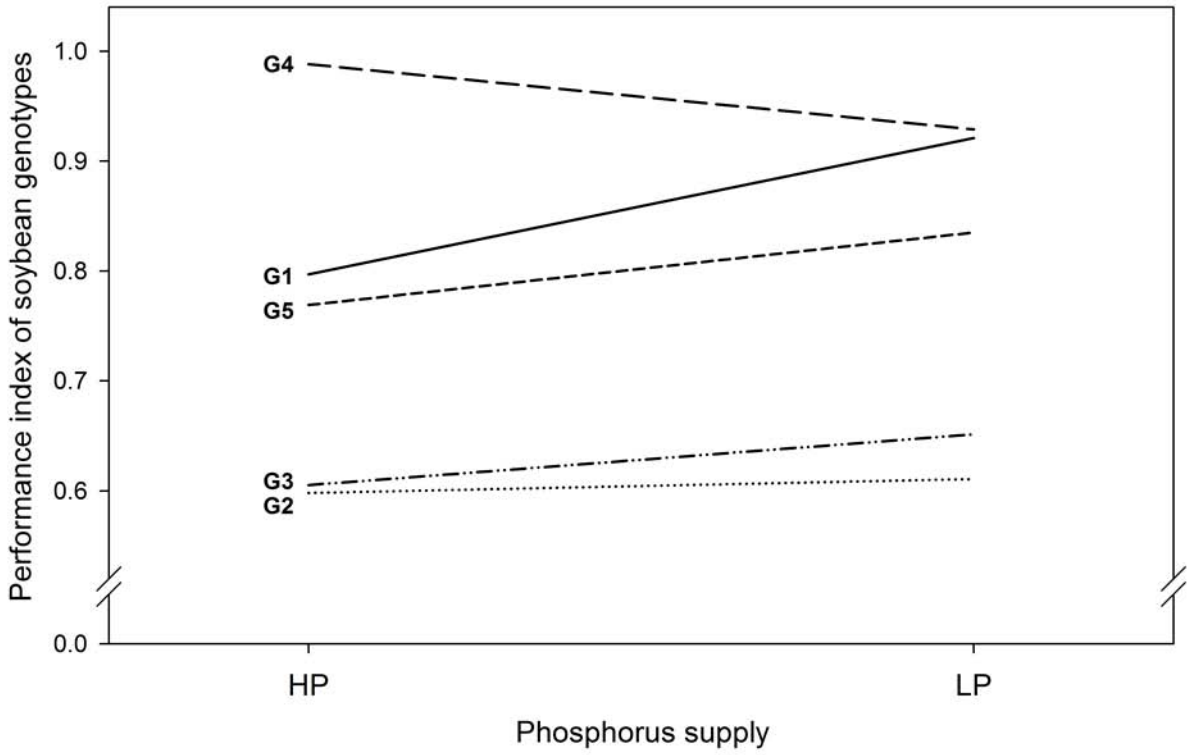

Figure 7: Relative performance of soybean genotypes based on their observed root phenes in the layered pot experiment. G1, G2, G3, G4, G5 represent soybean genotypes ES Mentor, Chyazni No 2, Kyoto-Soy, Riede 525, and Amurskaja Zlutozelená, respectively. HP and LP denote high and low soil P levels.

The results of Figure 7 showed that G4 was the highest-ranking genotype (PI=0.99) under HP followed by G1 and G5. Under LP treatment the performance of G1 was intensely increased, so that G1 and G4 achieved a similar performance index. Genotype 5 showed an increase in PI in relation to reduced soil phosphorus supply. G2 and G3 were the lowest ranking genotypes under both HP and LP treatments. To evaluate the overall relative performance of a genotype based on the observed root traits in soil-grown plants (RL, RD, RDM, RS-R, SRL, and BRNO), a performance index (PI, ranging from 0 to 1) under both low and high P levels was calculated as:

$$PI = \sum_{i}^{N} (V_i/V_{max}) \Big/ N$$

Where PI is the genotype-specific performance index (0-1), V_i is the genotype-specific trait value, V_{max} is the corresponding maximum value achieved by one of the genotypes, and N is the number of root-related traits, which was equal to six.

Total plant phosphorus uptake (PPUP)

As shown in Figure 8, the total average accumulated P at plant harvest (plant P uptake: PPUP) under HP treatment was 43.1 mg plant/1. In the LP treatment the PPUP decreased for the 54%. G4 and G5 had the highest P accumulation in the tissues in both P conditions, G3 the lowest one. G4 and G5 had accumulated total phosphorus higher than the control G1 in both P conditions. G4 has 18.6% and 9.9%, respectively for HP and LP, more P accumulation than the standard genotype G1.

P utilisation efficiency (PUtE)

The P utilisation efficiency (PUtE), expressed as the total amount of dry mass produced per unit of P taken up, was significantly affected by P treatment. Under high P treatment, genotypes had an average PUtE of 0.324 g DM mg^{-1} P and on the other hand, under low P condition the PUtE was increased to 0.363 g DM mg^{-1} P. The soybean genotypes had also different PUtE. G1, G2, G3, and G4 showed similar PUtEs (average 0.356 g DM mg^{-1} P), but G5 seemed to be the least efficient genotype in biomass production per unit P taken up (0.292 g DM mg^{-1} P).

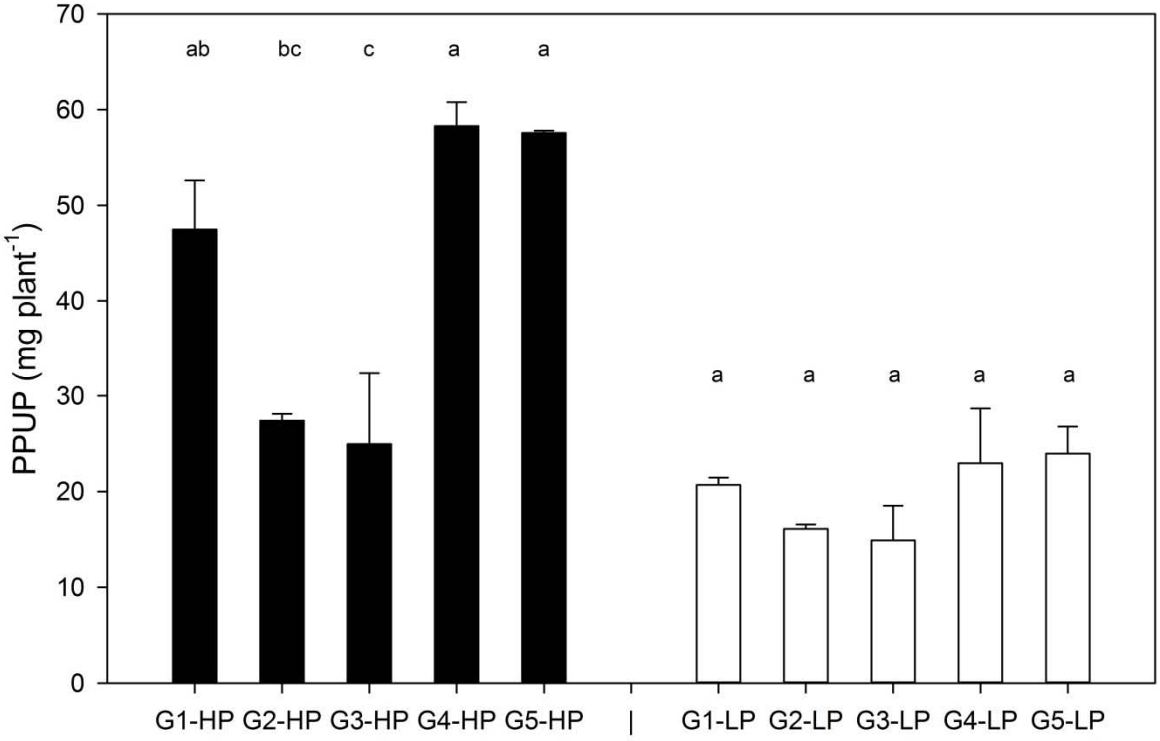

Figure 8: Total plant phosphorus uptake (PPUP) at anthesis of soybeans grown in a layered pot experiment. G1, G2, G3, G4, G5 represent soybean genotypes ES Mentor, Chyazni No 2, Kyoto-Soy, Riede 525, and Amurskaja Zlutozelená, respectively. HP and LP denote high and low soil P levels. Values are the means of three replicates ± standard error. Bars within a P treatment with the same letter are not significantly different (p=0.05).

Phosphorus uptake per unit of root length and root dry matter (PUP-RL) (PUP-RDM)

Genotypes were different also in P uptake per unit root length and root dry matter (PUP-RL in mg P m/1 root length; PUP-RDM in mg P g/1 root dry matter). There were no significant differences in PUP-RL and PUP-RDM at P treatment level. As showed in Figure 10, G4 was the least efficient genotype for PUP-RL and for PUP-RDM (0.255 ± 0.002; 23.63 ± 1.204).The standard genotype G1 had similar values with G4 and G2, G3 and G5 were more efficient for PUP-RL and for PUP-RDM.

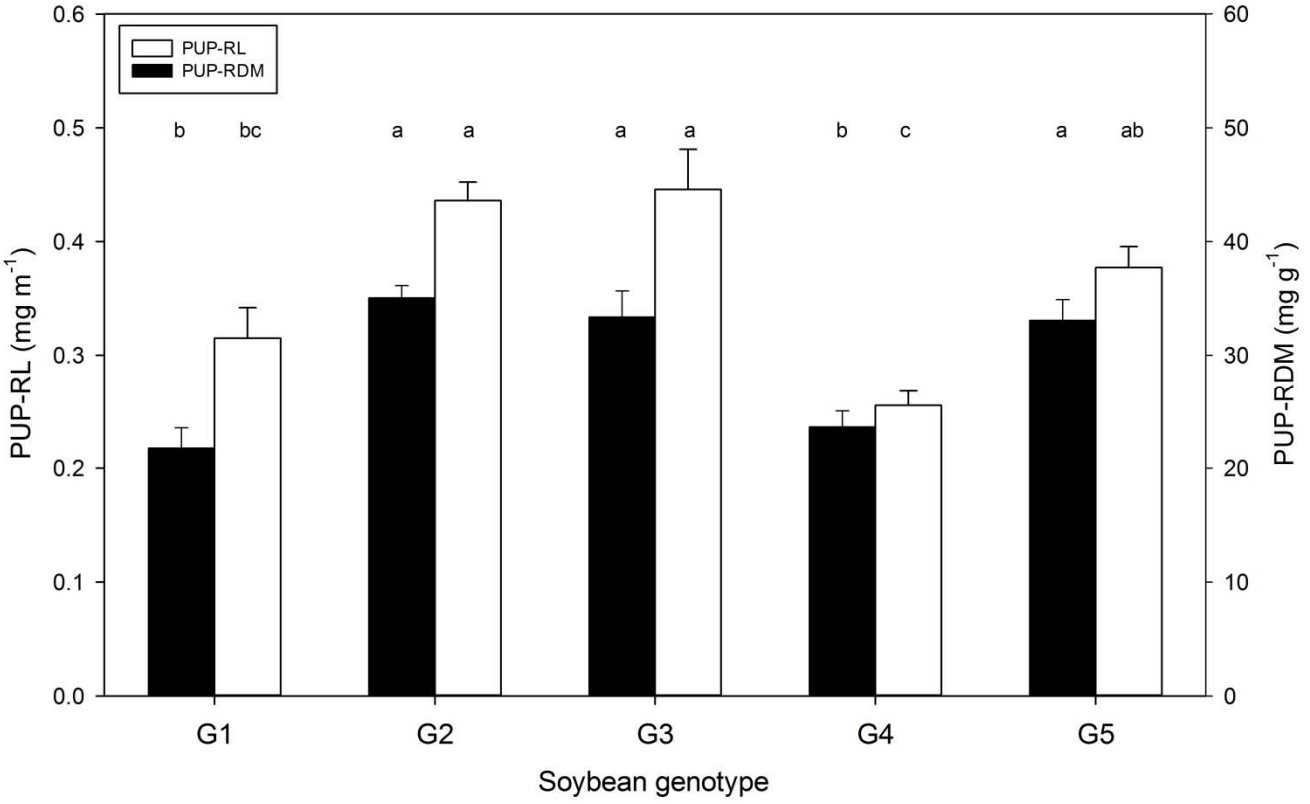

Figure 9: Phosphorus uptake per unit root length (PUP-RL) and root dry matter (PUP-RDM) in soybean genotypes. G1, G2, G3, G4, G5 represent soybean genotypes ES Mentor, Chyazni No 2, Kyoto-Soy, Riede 525, and Amurskaja Zlutozelená, respectively. HP and LP denote high and low soil P levels. Values are the means of three replicates ± standard error. Bars for PUP-RL and PUP-DM with the same letter are not significantly different (p=0.05)

6. Discussion

Research of new strategies to enhance phosphorus efficiency of legume crops, especially soybean, can be one of the goal of agriculture in the next decades and can bypass the potential lack of phosphate fertilizers in the future, exploiting soil resources in a more sustainable way.

An improved P efficiency of new varieties can reduce the amount of fertilizers and the waste of them through erosion, optimizing in a low input way the P plant demand of crops. Phosphorus acquisition by legume plants is influenced by root architecture and specific root traits. The immobility of this element in soil causes different availabilities in soil horizons; topsoil foraging is one of the strategies that can help plants to acquire more phosphorus, a relevant limiting factor that influences harvests. This study wants to investigate in soybean plant the genotypic variations of root architecture and different morphological root adaptions to acquire more phosphorus and to quantify its accumulation at whole plant level.

The 5 soybean genotypes included in this experiment were ES Mentor, Chyazni No 2, Kyoto-Soy, Riede 525, and Amurskaja Zlutozelená. They were selected from a range of forty soybean genotypes, tested in a previous experiment (Trittinger S. 2014). The range of soybean seedlings was scanned and analyzed through root digital images, observing and clustering the root development variations within them. Through the pouch system technique and genotype root screening, five cluster groups were identified. Each group contained soybean genotypes with root traits similarities based on basal root growth angle (BRGA), basal root number (BRNO), root length (RL-A) and root diameter (RD-A) at topsoil level. Assuming that P is immobile in the soil and more available in the first horizons, a topsoil foraging soybean genotype model should have a shallower BRGA, a greater BRNO, a higher RL and a thinner RD to acquire more P.

Topsoil foraging is influenced by traits such as the root growth angle, the development of adventitious roots, the number of axial roots, and the dispersion of lateral roots (Lynch 2011). In Trittinger's experiment there was no soybean genotype that had all these high performance root characteristics. Every cluster group had some of these features that could increase P acquisition. For example, Group 2 had a shallower BRGA and a smaller RD and Group 1 a higher BRNO and RL-A, compared to the others genotypes.

However, group 2 had a scares BRNO and RL-A and Group 1 showed an average BRGA and a higher RD-A. In the cluster groups 5 representative genotypes were selected for the soil layered experiment.

These five genotypes, selected for one or more low P adapted root traits, were evaluated in PVC columns filled with a growth substrate composed by sieved field soil and sand. Phosphorus availability differed in the two treatments, creating high P topsoil condition in the first one and a whole low P availability for the second one. Plants grown and tested in field soil with predetermined P available concentrations simulated topsoil field conditions in a glass-house experiment. The tubes allowed a view of the root development in the soil horizons with a minimal contamination between the different layers. A cause of the moderate dimensions of the pots (25 cm of diameter), the natural lateral growth of the roots was impeded by the wall of the tubes. At root harvest moment, a lot of lateral branches reached the inner wall surface, growing down in a right angle shape. This disturbance was a limit for an objective observation of the natural development of a root system. The average root diameter of this experiment was similar to those values reported for field conditions of soybean by Fenta et al. (2014) (0.43-0.49 mm) and Mahanta et al. (2014) (0.490-0,522 mm), but the average diameter of lateral branches in the experiment of Fenta et al. (2014) was 2.1-4.4 mm; quite higher, compared with our study where the corresponding root length in this diameter class was on average just 20 cm. Probably this 20 cm root length was counted with the tap root. The lateral axes in this PVC experiment were thinner than those grown at field conditions. Environmental factors like soil temperature, water content and different textures influence the soil strength that determines the plant capacity to root elongation. High mechanical impedance reduces the rate of root elongation with an increase of root diameter (Gregory 2006; Rich and Watt 2013). The soil strength in the growth soil mixture was not analyzed, but a soft texture of the substrate, composed by Chernozem soil and sand, and stable water content in the tubes, avoiding any water stress influences in plant growth, made lower soil strength. Probably was for this reason that the root diameter of axial branches was smaller compared with the values of Fenta et al. (2014). Furthermore root washing, separating soil particles from plant tissues, and root sampling destroyed the original spatial root arrangement of the plant and its gravitropic characteristics in the substrate. Alternative techniques like rectangular root chambers with Perspex sides of sufficient sizes combined with a pin board (Manschadi et al. 2008) can be more precise for root architectural analysis.

For the selection of low P efficient root traits and the following development of new P efficient soybean varieties, these traits should be ideally visible and expressed at an early stage like seedlings stage, and such peculiarities had to be kept later in the next

developmental stages, determining the development of the root system also at adult stages (Manschadi et al. 2006).

This experiment wanted also to investigate the potential correlation between root phenes observed at seedling stage in the pouch system and phenes of the same genotypes grown till flowering stage in substrate -filled PVC columns. In our experiment the numbers of hypocotyl-born roots, basal and adventitious roots were for each genotype higher than in the pouch system. G2 was in both experiments the genotype with the lowest BRNO. On the other hand G5 showed, in the pot experiment, similar values with others genotypes, while in the pouch experiment there was a marked difference within them.

The genotypic variation in number of adventitious and hypocotyl roots continues to be expressed after the formation of basal roots at seedling stage. The results of that experiment showed that genotypes of the same cluster group with similar root characteristics at seedling stage, didn't exhibit the same root phenes and P uptake in later developmental stages at grown- soil condition in PVC columns. G1 and G2 were in the same cluster group with root traits similarities, but in the pot experiment these two genotypes showed different developments and opposite P performances without any correlation between the values of the two experiments in terms of root characteristics.

Also G3 and G4, which were clustered together in Group 2, had in layered experiment at flowering- early pods stage, very different root characteristics and P acquisition capacities. For further analysis of correlation between pouch system and soil-grown experiment, G1, G4 and G5 showed similar total plant biomass production and accumulation.

G5 exhibited more root length in the upper section of the pouch system than G1 and G4, and in the pot experiment G5 had the 62% of the total root length in the first layer A, while G1 and G4 allocated in the first 20 cm of the substrate a lower total root length, 54% respectively on average.

In any case, a root length comparison between the 2 experiments can't be reliable because of several influences during plant growth, like translocation of assimilates to the roots, root growth and root branching during later developmental crop stages.

Root diameter (RD) was one of the root characteristics which significantly differed between the experiments. G4 exhibited the thinnest root diameter both in the pouch system and in the pot experiment. In soil condition, however, the diameter was lower, with thinner roots compared with the RD of G4 in Trittinger's experiment. This difference can be caused by root branching in soil, which decreased the average RD at whole adult plant level.

Compared to G1 and G5, the fraction of root length of G4 in PVC columns was lower in all diameter classes except for RDC1 (<0.2 mm). That means that the specific root length (SRL) of G4 was higher in comparison with the SRL of the others soybean genotypes. In a study at field conditions, two soybean genotypes and their 88 recombinant inbred lines (RILs) (Ao et al 2010) exhibited SRL values ranging from 0.08 to 51.38 m $g^{-1.}$

In another study, Rieger and Litvin (1999) measured SRLs of 45.9 to 63.1 m g^{-1} in soybean plants harvested after 2 months of growing. In this experiment G4 reached values of SRL around 92.53 m g^{-1}, having very thin roots and denoting as genotype a high efficiency in terms of root length per unit of root dry mass.

In the experiment the RD of G5 was thinner than at seedling stage maybe because of root branching. The specific root length of G4 (SRL, Table.4) is higher than in the others genotypes because the G4 plants compensated the very thin diameter of their roots, by developing the roots in length. Differences in root diameter were influenced by the size of cortical cell and the number of cell files across the cortex as well as the thickness of stele or xylem vessels (Rieger and Livin 1999). In rice, variation in RD among different root types has been associated with the number of cortex cell layers, with the thin lateral branches having no cortical cells and the thick crown roots exhibiting 10 cortical cell layers (Coudert et al. 2010).

Further investigations are needed to understand why G4 has such thin roots and if depends on the characteristics and number of cortex cells or/and on the stele diameter. The root system development needs different metabolic investments; these costs change towards the anatomical structure of the system. Furthermore soil exploration and the ability to acquire nutrients for maintenance and growth are influenced by the structure of the root system. Reduced number of cortical cells and greater cortical cell size substantially reduce root respiration, while greater cortical cell size reduces the metabolic investments for soil exploration, as larger cells have proportionally more cell volume for vacuole, which has less N, P and contribution to respiration than cytoplasm (Lynch and Ho 2005, Lynch 2014). The anatomy of roots plays also an important role in the hydraulic conductivity for the acquisition of water. The diameter of xylem vessels is fundamental for water transport in plant, with larger vessels for a higher water uptake and thinner vessels with less conductivity (Lynch et al. 2014). The radial hydraulic conductivity is inversely related to RD and cortex, reminding that thinner roots or roots with thinner cortex have higher hydraulic conductivity (Rieger and Litvin 1999). G4, thanks to his marked thin root system can acquire more P and nutrients and probably have more conductivity for water absorption.

The advantages for nutrients and water uptake in thinner roots can be also followed by the difficulties to penetrate soil because of the reduced thickness in hard soil textures (Clark et al. 2008; Rich and Watt 2013). Given that, G4 could have more problems to break and to penetrate into hard soil particles but recent studies in maize suggest that the root diameter in not the primary factor that influences the root biochemical properties and penetration abilities. Root anatomical phenes like cortex morphology and stele diameter can be relevant factors that play a key role in root bend and tensile strength than root diameter (Chimungu et al. 2015). Further studies are needed to understand the genotypic variation in the anatomical structure of soybean roots and its relation with root penetrability and biomechanics.

Big differences were founded in total root length (RL-T) between genotypes. G4 showed the greatest root length density (RLD, cm root cm/3) both with a greater root system vigour in the low P treatment and in the high P. G4 reached the maximum RLD with 1.86 cm cm/3 in the toplayer, while G1 and G5 have respectively 1.06 and 1.29 cm cm/3.

Studies declare that the root length density (RLD) in the first soil horizons of temperate cereals is 5-10 cm cm/3 and 1-2 cm cm/3 for crops like legumes (Gregory 2006). Mahanta et al have reported that the RLD of soybean plants grown on field conditions is 0.86 cm cm/3. In the experiment, Genotype 1 and 2 have a higher value in RDM than those measured by Mahanta et al. G4 gives better performances in terms of root growth and soil exploration. Moreover between G4 and G5 there are big differences in root length and root diameter but the P concentration in the plant tissues in both genotypes at harvest time is similar (Fig.8).

The similar P uptake values indicate that G5 is more efficient in P acquisition per unit of C investment in the root system. The results in PU per unit of root length and dry mass suggest that G5 has a better P acquisition compared to G4 and G1 (Fig.9). The phosphorus uptake per unit of root length is influenced by different factors like root hairs density, root length, exudation of protons, phosphatases, mycorrhizal symbiosis and up-regulation of P transporters. On the other hand PU per unit of root weight is promoted by reduced root: shoot weight ratio, thinner root diameter and the presence of aerenchyma (Richardson et al. 2011; Oburger et al. 2011; Lynch 2011; Vandamme et al. 2013; Hunter et al. 2014).The reason why Genotype 5 is more efficient, needs further investigations. At rhizosphere condition, the mobilisation of P is facilitated by the plant release of carboxilates and protons. During the mobilisation process not only P but also others micronutrients like manganese (Mn) are involved. Lambers (2015) declares that screening

Mn leaf can be used to select genotypes with higher P efficiency at low P soil conditions. When the relationship in soybean plants between P and Mn is understood, manganese leaf screening can be a new low cost, efficient technique to select genotypes identified for a better P mobilisation capacity.

7. Future prospects

The genetic variation and richness in root characteristics of soybean genotypes is a high potential opportunity for breeding new varieties that will be requested from agriculture of the next decades. The variability in root plasticity, caused by external factors like soil and environment, makes the selection of traits and genotypes very complex. The mechanisms that take part to the increase of P acquisition by plants are different and involve several disciplines. For this reason such studies need an interdisciplinary approach to understand the whole plant- environment system. Not just topsoil foraging and some specific root traits play a key role in P efficiency, but also mobilization processes in the rhizosphere, mycorrhizal symbioses and root anatomy. Focusing just on specific P efficient root traits is dispersive because such traits are connected and influenced by several environmental, physiological and genetic factors.

Lynch (2005) declares that just few studies about the effect of isolated traits on P efficiency are available nowadays. Because of the large genetic variation within and among species, it is difficult to make general statements on root traits for P efficiency. Lynch (2005) supposes that agro-ecological studies of crops where traits can be isolated through crossing might be more successful than the comparison of wild plants.

Marker assisted breeding techniques will provide new opportunities for research in the next years. In the last decade, new studies have brought interesting discoveries for sequencing and molecular marker technologies. Consequently a huge range of genomic markers for plant crops are already available (Vinod and Heuer 2012). Unfortunately literature on QTL analysis for P efficiency in soybean remains scarce (Wang et al. 2010).

A study by Liang et al. (2010) identified putative loci in the soybean genome for root traits and P efficiency. Genetic markers are a promising tool in plant breeding for nutrient efficient crops since measurements of root characteristics in the field are difficult and costly (Lynch 2005).

The use of crop models is a promising approach in the pre-selection process of nutrient efficient varieties to overcome problems caused by the interactions between genotype, environment, and management practices (Manschadi et al. 2014). Computer models, have already been used to simulate root characteristics like root gravitropism, inter-root competition or nutrient uptake by root hairs (Ge et al. 2000, Leitner et al. 2010). Devau et al. (2010) used a mechanistic model to evaluate root-induced changes in soil P availability.

8. Conclusions

The results of the experiment reveal that root architectures of similar soybean genotypes at seedling stage vary in P acquisition capacity at adult stage, because plants modify their own root growth and root: shoot ratio during their development from seedling to flowering time. The observation and the selection of P-adapted genotypes cannot be accomplished through seedlings in pouch system. There are many root- shoot interferences during plant growth, which change the primordial root system design of seedlings and influence P-acquisition capacity in the adult developmental stages.

However, a great variation in root characteristics exists between genotypes, having an enormous genetic potential for future breeding programs (Zhao et al.2004, Ao et al.2010). The 2 genotypes of the experiment that are interesting for P- efficiency compared to the standard genotype 1 are G4 and G5. Riede (525) G4 has an explorative root system with very thin roots and similar P- utilisation efficiency of G1.

In a research program with two contrasting soybean genotypes and their F9-derived recombinant inbred lines (RILs), Wang et al. (2004) observe that root hair traits, including root hair density, average root hair length and root hair length per unit root, varied significantly among different genetic materials and that these variations were highly associated with P status in soybean plants. Root systems with a smaller root diameter and a larger surface area explore the soil more effectively, thereby improving P acquisition (Machado and Furlani 2004).The other genotype, G5 Amurskaja Zlutozelena, has the same P accumulation of G4 in the tissues, but with a less extensive root system. Exudation of organic acids into the rhizosphere has been proposed to increase P availability to the plant by mobilizing the sparingly soluble mineral P and, possibly, organic P sources (Dinkelaker et al., 1989; Jones and Darrah, 1994; Johnson et al., 1996). Dong et al. (2004) showed that soybean genotypes contrasting in P efficiency differed in the type and quantity of organic acids excreted from the roots under P stress, suggesting that organic acids might contribute to P uptake in P-efficient genotypes.

G5 has a higher P-uptake efficiency per unit root length and dry mass but a lower P-utilisation efficiency than G1 and G4.In both cases, for G4 and G5, the root- shoot ratio is lower compared to G1. These 2 P- efficient soybean genotypes show 2 different physiological strategies to increase P acquisition. The extended and dense root system of G4 is more explorative and has more soil: root surface to adsorb P (exploration strategy). The second way, with G5, is increasing the P- uptake per unit of root length, having a higher exploitation efficiency of a soil unit (exploitation strategy). Soybean plants react in

different ways to adapt in low P soil conditions. Further research is needed to understand the genetic expression and the physiological adaptation of a thin explorative root system and an exploitative P- uptake system, composed by different and contrasting root phenes. This experiment ended at flowering time; further experiments can investigate on root phenes and P- acquisition efficiency in more complex systems, studying nitrogen fixation and pods maturation influences on P acquisition and root development. The assessment of P efficiency in plants is very complex and needs an interdisciplinary approach. Experiments with plants grown under field conditions can give a wider understand with more details about P- efficient root phenes and its development. The use of crop models is a experimental approach in the pre-selection process of nutrient efficient varieties to overcome problems caused by the interactions between genotype, environment, and management practices (Manschadi et al. 2014).

Germplasm screening and trait discoveries are two key components in an interdisciplinary research framework aiming at enhancing nutrient efficiency in crops. Further experiments are needed to understand how soybean plants interact in agroecosystems to acquire more phosphorus. Field experiments could be useful, testing plants in natural low P soil condition till final developmental crop stages.

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10. List of figures

11. List of tables

11. Abstract

Phosphorus is one of the 17 mineral nutrients essential for plant growth. Phosphorus is classified as a major and primary nutrient, meaning that it is frequently deficient in agricultural soils and is required by crops in relatively large amounts, making P an important constraint to crop productivity. The identification of adapted genotypes that use soil P more efficiently is therefore considered as a strategy to withstand the probable lack of phosphate rocks and to exploit the natural P resources in the first horizons of the soils. The soybean is economically the most important bean in the world and is the most nutritious and most easily digested food of the bean family. The soybean is one of the richest and cheapest sources of protein The objective of this work was to test 5 soybean genotypes, contrasting in P acquisition. The genotypes were selected by a genetic evaluation in root architectural and morphological traits with relevance to P of 40 soybean genotypes. One of the five was a commercial soybean variety, used as control. Plants were grown in PVC tubes filled with air dried sieved field soil. Each genotypes was subjected to 2 different P treatments; one with a high P availability in the first 15 cm of the tube and the second with a total low P availability in the soil horizons. Plants were harvested after 64 day, at later flowering stage. Plant organs were dried, weighted and analyzed for the P content. Roots of the different tube layers were analyzed using WinRHIZO Pro and OpenGelPhoto 2a. The results showed 2 genotypes that absorb the same and more P than the control plants. One genotype using an explorative strategy, with a higher soil root surface and an extended and dense root system, the second one having an increased P- uptake per unit of root length, with a higher exploitation efficiency of a soil unit (exploitation strategy). Germplasm screening and trait discoveries are two key components in an interdisciplinary research framework aiming at enhancing nutrient efficiency in crops. Further experiments are needed to understand how soybean plant interact in agroecosystems to acquire more phosphorus.