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# THE ROLE OF SEEDS IN PHOSPHORUS-ACQUISITION OF SOYBEAN (*Glycine max*)

A dissertation submitted in partial fulfilment of the requirements for the degree of Dr.nat.techn. (Doktorat der Bodenkultur)

Submitted by:

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Vienna, November 2020

# Eidesstaatliche Erklärung

Ich erkläre eidesstattlich, dass ich die Arbeit selbständig angefertigt habe. Es wurden keine anderen als die angegebenen Hilfsmittel benutzt. Die aus fremden Quellen direkt oder indirekt übernommenen Formulierungen und Gedanken sind als solche kenntlich gemacht. Diese schriftliche Arbeit wurde noch an keiner Stelle vorgelegt.

Wien, November 2020

## Acknowledgement

I would like to thank the people, without whom I would not have been able to finish this research, and without whom I would not have made it through my PhD study.

The staff of Institute of Agronomy at University of Natural Resources and Life Sciences, Vienna, especially to my supervisor Univ.Prof. Dipl.-Ing. Dr.nat.techn. Hans-Peter Kaul, whose support allowed my studies to go the extra mile. And special thanks to Assoc. Prof. Dr. A. M. Manschadi, whose insight and knowledge into the subject matter steered me through this research.

I would also like to thank Dipl.-Ing. Stefan Peter Ryall, Tamara Kolodziej, Herbert Blaich, Markus Freudhofmaier, Craig Jackson, Dr. Aliyeh Salehi, Dr. Mansour Taghvaei, Dr. Hossein Mostafavi and Dr. Alireza Nakhforoosh for their assistance in measurements and technical support on my study. I am as well thankful to Nadia Sasani for always being there for me and supporting me empathically.

I am also especially grateful to my parents, who always encouraged me in so many ways. I always knew that you believed in me and wanted the best for me.

**Abstract**

The low phyto-availability of phosphorus in the soil is a major constraint to soybean production and therefore, looking for phosphorus efficient soybean varieties that can efficiently acquire and utilize the mineral P from the soil is an important breeding objective to secure and improve soybean production. Few studies have been published on the importance of seed weight or rather seed P content in soybean for P supply of soybean plants. However, there is still a need to study genotypic differences in how the root system and seed P content are involved in supplying P for young soybean plants. In order to evaluate the relations between seed size and seed P with shoot P uptake, two pot experiments were conducted under greenhouse conditions. In the first experiment (EXP1), 15 soybean genotypes with significantly different seed weights were grown in a 1:1 soil-sand mix (w/w) with a low P phyto-availability of 21.6 mg $kg^{-1}$ for 30 days. In the second experiment (EXP2), a greenhouse pot experiment with two P supply treatments – low P (LP) vs. high P (HP) with a P phyto-availability of 6.17 and 68.12 mg $kg^{-1}$ respectively – was undertaken, harvesting five genotypes of soybean differing in seed weight and shoot P uptake at 40 days after planting (DAP) based on the previous screening in EXP1. In EXP1, results demonstrated that at 30 DAP, shoot P uptake was significantly different between genotypes, and we found a strong, positive correlation between seed weight as well as seed P content and shoot P acquisition, shoot dry weight and total plant dry weight. The results also revealed that seed weight is stronger associated with shoot biomass than with root biomass. In EXP2, bivariate correlations of seed weight and seed P content with aboveground biomass or total plant biomass, as well as shoot P content showed that the early biomass accumulation is strongly governed by seed size and seed P content at LP. However, between-genotype variation in seed weight and seed P did not affect the root growth at 40 DAP. The seed weight and P content were both significantly and positively correlated with shoot P uptake both at LP and HP, nevertheless the correlation under LP was stronger. The correlations of root weight and root morphological traits with shoot P were insignificant. Furthermore, at LP the total shoot P uptake for each genotype was only slightly less than the average seed P for that genotype, which shows that theoretically mobilising the seed P content by itself is enough to meet the requirements of young plants until 40 DAP under P starvation conditions.

Key words: Phosphorus deficiency. Seed P content. Seed size. Seed weight. Root morphological traits. Early growth stages. Soybean genotypes.

**Kurzfassung**

Phosphormangel im Boden ist ein wesentliches Hindernis für die Sojabohnenproduktion. Daher ist die Suche nach phosphoreffizienten Sojabohnensorten, die den Phosphor aus dem Boden effizient aufnehmen und nutzen können, ein wichtiges Züchtungsziel, um die Sojabohnenproduktion zu sichern und zu verbessern. Es gibt nur wenige Studien zum Einfluss des Samengewichts bzw Phosphorgehalts der Samen für die Phosphorversorgung der Sojabohnenpflanzen. Es besteht also immer noch die Notwendigkeit, die genetische Variation zu untersuchen, um zu wissen welche Rolle das Wurzelsystem und der P-Gehalt der Samen bei der Versorgung junger Sojabohnenpflanzen mit P spielen. Um die Zusammenhänge zwischen Samengröße und Phosphorgehalt mit der Phosphoraufnahme des Sprosses zu untersuchen, wurden zwei Topfversuche unter Gewächshausbedingungen durchgeführt. Im ersten Versuch (EXP1) wurden 15 Sojabohnengenotypen mit signifikant unterschiedlichen Samengewichten in einer 1:1 Erde-Sand Mischung mit einer niedrigen Phosphorverfügbarkeit von 21,6 mg $kg^{-1}$ 30 Tage lang kultiviert. Im zweiten Versuch (EXP2) wurde ein Topfversuch im Gewächshaus mit zwei P-Versorgungsbehandlungen – P-Mangel (LP) vs. hohes P Angebot (HP) mit einer P-Verfügbarkeit von 6,17 bzw. 68,12 mg $kg^{-1}$ – durchgeführt. Hierfür wurden fünf Genotypen von Sojabohnen, die sich im Samengewicht und in der P-Aufnahme des Sprosses am 40. Tag nach der Saat unterscheiden, basierend auf dem vorherigen Screening in EXP1 ausgewählt. In EXP1 zeigten die Ergebnisse, dass am 30. Tag nach der Saat die Spross-P-Aufnahme zwischen den Genotypen signifikant unterschiedlich war, und wir fanden eine starke positive Korrelation zwischen dem Samengewicht sowie dem Samen-P-Gehalt und der Sprossphosphoraufnahme, dem Spross-Trockengewicht und dem Gesamttrockengewicht der Pflanze. Die Ergebnisse zeigten auch, dass das Samengewicht mit der Sprosstrockenmasse stärker verbunden war als mit der Wurzeltrockenmasse. In EXP2 zeigten bivariate Korrelationen von Samengewicht und P-Gehalt der Samen mit oberirdischer Biomasse oder Gesamtpflanzenbiomasse sowie Sprossphosphorgehalt, dass die frühe Biomassebildung stark von der Samengröße und dem Phosphorgehalt der Samen bei LP abhing. Die Variation des Samengewichts und des Samen-P zwischen den Genotypen hatte jedoch keinen Einfluss auf das Wurzelwachstum am 40. Tag nach der Saat. Das Samengewicht und der Phosphorgehalt waren sowohl bei LP als auch bei HP signifikant und positiv mit der Phosphoraufnahme des Sprosses korreliert, allerdings war die Korrelation unter LP stärker. Die Korrelationen von Wurzelgewicht und der wurzelmorphologischen Merkmale mit dem P im Spross waren nicht signifikant. Darüber hinaus war bei LP die Gesamtaufnahme von P im Spross für jeden Genotyp nur ein wenig geringer als der durchschnittliche Samen-P-Gehalt für diesen Genotyp, was zeigt, dass die theoretische

Mobilisierung des Samen-P-Gehalts allein ausreicht, um die Anforderungen junger Pflanzen unter mangelhafter Phosphor-Versorgung zu erfüllen.

Schlüsselwörter: Phosphormangel. Samen-P-Gehalt. Samengröße. Samengewicht. Jugendentwicklung. Sojabohnen-Genotypen.

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## 1. Introduction

Soybean (*Glycine max* (L.) Merrill) is a key crop in fulfilling the needs of human nutrition as well as animal feed with regard to oil, protein, calories and by-products. More than 140 years ago, Haberlandt (1877), who was professor at the University of Natural Resources and Life Sciences Vienna (BOKU), successfully introduced the soybean plant to Central Europe and discussed its cultivation for the first time. In reference to Liu (2012), Tani Lee *et al*. (2016) and Tewari, *et al*. (2016), soybean is a very important crop due to its seeds, which have the highest protein concentration, and also one of the highest oil concentrations among legumes, containing carbohydrates and other minerals as well. As reported by Kolmanič (2017), ever since its cultivation was begun in the Pannonian Basin, its regional supply has not kept up sufficiently with the demand.

The dilemma of phosphorus (P) in agriculture with regard to its status as a finite resource has been already mentioned extensively and thoroughly discussed in a research paper by Gilbert (2009). The low phyto-availability of phosphorus in the soil is a major constraint to soybean production and therefore, looking for phosphorus efficient soybean varieties that can efficiently acquire and utilize the mineral P from the soil is an important breeding objective to secure and improve soybean production Wang *et al*. (2010). Manschadi *et al*. (2014) described that P-efficient crops and genotypes show greater P acquisition efficiency with lower soil-P requirements and also mentioned the necessity of finding or developing them, which would minimize the excessive use of P fertilisers, resulting in a better P-balance and reducing the losses and subsequent environmental side effects associated with excessive use of P regarding runoff, leaching and eutrophication. Rose *et al*. (2013) also proved that some crops are able to grow at lower P concentration in the biomass (2 mg P $kg^{-1}$) which can be as considered as low-P tolerant (with regard to P utilisation efficiency). On contrary, the plants which cannot perform well under low P phyto-availability will be considered as P-sensitive. It has been also a concern for long to provide germplasm with efficient P uptake and utilization Zhang *et al*. (2005).

According to the Austrian Agency for Health and Food Safety (AGES), any P content between 26-46 mg $kg^{-1}$ CAL-P in a substrate is classified as “low”, whilst P contents lower than 26 mg $kg^{-1}$ CAL-P will be considered as” very low”. *Vandamme et al*. (2016) in their first experiment on soybean used 60 mg $kg^{-1}$ and 115 mg $kg^{-1}$ for LP and HP respectively (P was added at a rate of 60 mg P $kg^{-1}$ sand for LP and 115 mg P $kg^{-1}$ sand for HP by mixing the hydrous ferric oxide-coated sand with a $KH_2PO_4$ solution), and in their third experiment on soybean they used P-deficient farming soil of 2.2 mg P $kg^{-1}$ amended with nutrient solutions containing 75 mg P $kg^{-1}$ (low P) and

400 mg P $kg^{-1}$ (high P) to induce a milder stress at LP. In another experiment by Wang *et al*. (2010) on soybean, the low P supply level to induce the P deficiency stress was as low as 12.60 mg P $kg^{-1}$. As indicated by Sandaña, and Pinochet (2014), we know that the P uptake in soybean is not as efficient as with other legumes like lupin, which took up on average 2.6 times more P per unit root length than soybean.

Soybean is a large seeded plant with big cotyledons and accordingly considerable seed P reserves. Veneklaas *et al*. (2012) reported that the total P concentration in the seeds of soybean is around 7.5 mg $g^{-1}$ fresh mass (FM), which is up to 2.5 and 1.5 fold higher than the seed P concentration in rice (*Oryza sativa*) and common bean (*Phaseolus vulgaris*), respectively.

In reference to the findings of Vandamme *et al*. (2016), *Mebrahtu et al*. (1997) and Smith and Camper (1975), it has been hypothesized that seed P maintains the growth of the young soybean plants beyond the seedling stage especially under low P phyto-availability of soil, and it also improves the plant establishment, increases the later productivity and enhances the shoot P uptake through earlier access to the exogenous P resources by a faster root establishment.

Mean kernel weight as an index which represents the seed size, is generally consistent under environmental stresses including P deficiency stress, and it is strongly controlled by genes as shown e. g. in wheat by Shearman *et al*. (2005) and Masoni *et al*. (2007), in canola by Manoharan *et al*. (2007) and in chickpea by Valimohammadi & Tajbakhsh (2007). Furthermore Yan *et al*. (1995) suggested that heavier seeds with higher seed P content are inheritable and could be considered as an adaptation trait developed by plants in response to low soil P phyto-availability.

It is also already evident that soybean cultivars differ genotypically with regard to P uptake and therefore, screening for P efficient soybean varieties that are capable of efficient acquisition of the mineral P from the soil will be necessary to improve soybean production as emphasized by Wang *et al*. (2010). Blair (1993) pointed out that there are many characteristics whereby crops can tolerate low P stress. Among them one can mention the differences in root branching patterns and the rate of uptake per unit root length, root extension rates, or root dry weight, root:shoot and shoot:grain allometry and the minimum required tissue P concentration for plants to function. Of course, most of those traits can somehow be altered by traditional or biotechnological breeding techniques.

Nevertheless, not all of the sources for these variations in P acquisition and use efficiency are fully understood. E.g. it is not known to which degree the seed P content would affect the shoot biomass and the shoot P content. Liao and Yan (1999) indicated that seed size is closely related to

phosphorus use efficiency in common bean. Despite the fact that dependency of plants on seed P gradually fades over time after emergence, *Vandamme et al*. (2016) suggested that in a large seeded crop like soybean its effect will drag out beyond the seedling stage.

## 2. Hypothesis and research questions

Knowing that under low P supply conditions, the young soybean plants are highly relying on seed P content within the early growth stages, we hypothesised that screening soybean genotypes of different seed sizes for early biomass and P accumulation will help to prove, that those genotypes which have relatively higher seed weight and seed P content, can potentially provide a reliable P supply to sustain a better early biomass accumulation in the shoot and root which will be later in favor of the total P uptake. This could be hypothetically by mobilising the P from the seed to the shoot or by investing it in the root parts for establishing a better root system and subsequent P uptake from soil. Overall, in the present study we have hypothesized that in soybean, seed weight or rather seed P content will positively affect the shoot and root growth and P uptake up to anthesis particularly under low P phyto-availability conditions of the soil.

Based on these hypotheses, the objective was to find out potential genotypic differences in the shoot P uptake among soybean cultivars with contrasting seed P at 30 to 40 DAP and also to determine the associations between seed P and also seed size with early shoot and root biomass, shoot P uptake and root traits under moderately to severely low P phyto-availability in contast with high soil P supply.

The results may provide breeders with selection targets in order to breed soybean for higher biological productivity under conditions of low phosphorus phyto-availability. In detail the following hypotheses were tested:

- In a set of soybean genotypes that differ in seed size, seed P content differs too and is closely (linearly) related to seed size.
- Seed P concentration, anyhow, is significantly variable in that set soybean genotypes, and it shows a slightly negative correlation with seed size.
- Shoot, root and total dry matter of plants at 30 and 40 DAP are also different, and all traits are closely (linearly) related to seed size.
- Shoot P content of plants at 30 and 40 DAP is different and closely (linearly) related to seed P content. The correlation to seed P concentration is less close.
- Shoot:root ratio differs significantly between genotypes during that early phase of plant development.

- The absolute amount of shoot P content is a linear function of seed P content with an intercept close to 0 and a slope slightly less than 1, reflecting the root P content and residual P in seed tissue.
- Differences between genotypes in root traits (i.e. total root length, root surface area) may exist, but they do not contribute significantly to explain differences in shoot P content, as root P uptake plays no important role during that early phase of plant development.

## 3. Materials and methods

### 3.1. Experimental Site and Conditions

A pot experiment was carried out in the greenhouse at the UFT Campus of BOKU in Tulln at the average temperature of 21.6 °C / 15.2 °C for day and night in EXP1 and 22 °C / 14 °C in EXP2. The RH (Relative Humidity) was 42.6% in EXP1 and 44% in EXP2. The lights were set to 14 h day / 10 h dark with 380 µmol photons $m^{-2}$ $s^{-1}$ provided by 4 metal-halide Phillips lamps (each 400 W) which hung 100 cm above the soil surface. The EXP1 was laid out in completely randomized design (CRD) with 8 replicates. EXP2 was also laid out in CRD but with 6 replicates.

### 3.2. Germplasm

Seeds of 15 varieties of soybean with different 1000 seed weight were provided by BOKU Division of Plant Breeding (Table 1). In order to avoid variations within the seedlings of the same genotype, only seeds of visibly the same size, same health appearance from a determined seed weight range (variation of seed weight within each genotype less than 1.6%) were chosen as the representatives of each genotypic population. Seeds of the genotype ES Mentor were used as a well-known reference and planted in both experiments as control.

In EXP2, three genotypes that accumulated the highest P content within a range of shoot biomass, which was not significantly different until 30 DAP in EXP1, namely Zolta Przebedowska, Riede 525 and Ford as well as the most vigorous genotype with highest biomass accumulation and highest shoot P concentration, i.e. Kyoto-Soy (G471), were selected for EXP2.

3.3. Plant growth substrate

In EXP1, the potting substrate was a 1:1 soil-sand mix (w/w). The soil that was used in this experiment was collected from an agricultural field at Bruck an der Leitha, Lower Austria, with previous cropping history of potato**.** Soil organic matter (SOM) was 2.36%, which has been measured by dry combustion using an elemental analyser (CNS-2000, LECO Corp., St. Joseph, USA) at 650°C, soil pH was 6.3, measured in a 1:2.5 (w/v) soil to 0.01 mol/L $CaCl_2$ suspension after 13 h, using a MultiLine® WTW 3420 glass electrode.

Average soil cation exchange capacity (*CEC*) was 15.3 *cmol* $kg^{-1}$ at the abovementioned pH and after adding sand it reached 10.2 *cmol* $kg^{-1}$. The measured plant bioavailable P concentration of the pure soil using the calcium acetate lactate (CAL) extraction of Schüller (1969) was 43.2 mg $kg^{-1}$, which according to the Austrian Agency for Health and Food Safety (AGES) is classified as "low". The potting mix was fertilized in EXP1 with 480 mg Compo Floranid NK in each pot (14% N, 19% $K_2O$, 3 % MgO, 11 % S, 0.02 % B, 0.01 % Cu, 0.5 % Fe, and 0.01 Zn) equivalent to a final concentration of 21.6 mg $kg^{-1}$ P, 33.6 mg $kg^{-1}$ N, 37.85 mg $kg^{-1}$ K, 4.34 mg $kg^{-1}$ Mg, 26.40 mg $kg^{-1}$ S, 0.05 mg $kg^{-1}$ B, 0.02 mg $kg^{-1}$ Cu, 1.2 mg $kg^{-1}$ Fe and 0.02 mg $kg^{-1}$ Zn .

In EXP2, the potting substrate was a 1:6 soil-sand mix (w/w). The soil that was used in this experiment was also collected from the same agricultural field as mentioned above. Average soil cation exchange capacity (CEC) for the potting substrate after adding the sand reached 6.2 cmol $kg^{-1}$. The measured plant bioavailable P concentration of the prepared substrate for LP and HP was 7.20 mg $kg^{-1}$ and 69.15 mg $kg^{-1}$ (CAL-P), which according to the Austrian Agency for Health and Food Safety is classified as "very low" and "sufficient", respectively.

An overview of pure soil and potting mix characteristics has been presented in Table 2. The nutrients were applied by the means of fertigation. A modified 20% strength Epstein nutrient solution as suggested by Epstein and Bloom (2005) was used, and the macronutrients were supplied differently for HP and LP pots. In this experiment the two levels of soluble P as well as other nutrients have been supplied on a regular basis (100 ml of the final solutions per day for the first 30 out of 40 days) and as evenly as possible to the soil surface using 100 ml mechanical pipettes to reach the desired concentration of elements in the potting substrate after applying the 3 liter of final solution. The plants were also watered with deionized water to fulfill their water requirements. A brief description of the supplied nutrients is presented in Tables 3, 4 and 5.

Table 1. Characteristics and origin of the soybean germplasm used in the experiments.

| code | Genotype | Seed source[a] | Maturity type[b] | 1000 seed weight (g) |
|---|---|---|---|---|
| G1 | Fruwirths Schwarze | SOJA 53/88 | n.a. | 154 |
| G2 | Zolta Przebedowska | SOJA | 00 | 227 |
| G3 | Gatersleben 36 | SOJA | n.a. | 196 |
| G4 | Brillmeyer Giesenska | SOJA | n.a. | 177 |
| G5 | Riede 525 | SOJA | II | 182 |
| G6 | Amurskaja 41 | SOJA | II | 147 |
| G7 | Ford | PI 548562 | III | 200 |
| G8 | Chyazni No 2 | SOJA | n.a. | 219 |
| G9 | Amurskaja Zlutozelená | SOJA | n.a. | 152 |
| G10 | Plaska Zlta Obravska | SOJA | 0 | 215 |
| G11 | ES Mentor | AUT | 00 | 206 |
| G12 | Christine | AUT | 00 | 148 |
| G13 | GH8X-8 | AUT | 00 | 199 |
| G14 | Kyoto-Soy (G471) | JPN | 0 | 304 |
| G15 | GNN2X-111-15 | BOKU | 000 | 146 |

[a] SOJA: Leibniz Institute of Plant Genetics and Crop Plant Research (IPK Gatersleben, Germany)
PI: USDA Soybean Germplasm Collection (USDA-ARS, Beltsville, MD, USA)
JPN, AUT, and BOKU: Seeds received from Japan, Austria, and from the soybean breeding program at BOKU University, Vienna, Austria, respectively.

[b] n.a.: not assigned to a maturity group

Table 2. Overview of pure soil and potting mix characteristics in EXP1 and EXP2.

| | Pure Soil (without sand) | 1:1 soil-sand mix (w/w) | 1:6 soil-sand mix (w/w) for LP | 1:6 soil-sand mix (w/w) for HP (EXP2) |
|---|---|---|---|---|
| measured P content (mg | 43.2 | 21.6 | 6.17 | 68.12 |
| Soil organic matter (SOM) | 2.36% | 1.2% | 0.34% | 0.34% |
| CEC (cmol $kg^{-1}$) | 15.3 | 10.2 | 6.2 | 6.2 |

Table 3. Composition of macro nutrients in one-fourth strength nutrient solutions for HP pots.

| Salt | Target nutrient | Nutrient concentration (ppm) | Nutrient in 3 l of irr. water (mg) | Concentration of the applied nutrient in the substrate (mg |
|---|---|---|---|---|
| Provided | N | 196.09 | 588.28 | 196.09 |
| $KNO_3$ | K | 273.69 | 821.06 | 273.69 |
| $Ca(NO_3)_2.4H_2O$ | Ca | 100.20 | 300.59 | 100.20 |
| $NH4H_2PO_4$ | P | 61.95 | 185.84 | 61.95 |
| $MgSO_4.7H_2O$ | Mg | 72.92 | 218.75 | 72.92 |
| $(NH_4)_2SO_4$ | S | 96.20 | 288.59 | 96.20 |

Table 4. Composition of macro nutrients in one-fourth strength nutrient solutions for LP pots.

| Salt | Target nutrient | Nutrient concentration (ppm) | Nutrient in 3 l of irr. water (mg) | Concentration of the applied nutrient in the substrate (mg |
|---|---|---|---|---|
| Provided | N | 182.09 | 546.26 | 182.09 |
| $KNO_3$ | K | 273.69 | 821.06 | 273.69 |
| $Ca(NO_3)_2.4H_2O$ | Ca | 100.20 | 300.59 | 100.20 |
| $NH_4H2PO_4$ | P | 0 | 0 | 0 |
| $MgSO_4.7H_2O$ | Mg | 72.92 | 218.75 | 72.92 |
| $(NH_4)_2SO_4$ | S | 112.23 | 336.68 | 112.23 |

Table 5. Composition of micro nutrients in one-fourth strength nutrient solutions for all pots.

| Salt | Target nutrient | Nutrient concentration (ppm) | Nutrient in 3 l of irr. water (mg) | Concentration of the applied nutrient in the substrate (mg $kg^{-1}$) |
|---|---|---|---|---|
| KCl | Cl | 1.77 | 5.32 | 1.77 |
| $H_3BO_3$ | B | 0.27 | 0.81 | 0.27 |
| $MnSO_4.H_2O$ | Mn | 0.88 | 2.64 | 0.88 |
| $ZnSO_4.7H_2O$ | Zn | 0.20 | 0.59 | 0.20 |
| $CuSO_4.5H_2O$ | Cu | 0.16 | 0.48 | 0.16 |
| $(NH_4)_6Mo_7O_{24}$ | Mo | 0.17 | 0.50 | 0.17 |
| Fe-EDTA | Fe | 5.03 | 15.08 | 5.03 |

### 3.4. Set up and execution of experiments

In EXP1, 1700 g of the potting mix and in EXP2, 2700 kg of the potting mix has been put into 4 liter (19 cm diameter by 20 cm height) plastic pots. Subsequently, four seeds were directly placed on the soil surface in each pot in a triangle arrangement with one in the middle and covered by a 300 g (3 cm column) of the same potting mix. Then they were irrigated immediately using deionized water to the saturation point. After that, they were left alone to let the soil water content go down to about 75% field capacity (FC) and maintained at that level by taking soil samples 4 days after watering for each pot. The samples were weighed, dried and weighed again to determine the gravimetric soil water content. The weight of the pots at about 75% FC was recorded and for the rest of the experiment the pots were weighed on a daily basis to get the amount of the water required to bring them back to 75% FC.

### 3.5. Harvest and measurements

The phenological development stages of soybean plants were documented according to BBCH scale as used by Munger *et al*. (1997), two times a week (Fig. 28). The plant shoots were harvested 40 days after planting by cutting them from the soil surface to get the aboveground biomass. The leaves were dissected from petioles as a separate sample fraction and measured for leaf area (LA) using the LI-COR LI-3100 leaf area meter. Then leaves as well as stems were oven-dried at 65°C for 72 hours to determine the dry matter (DM). The roots were washed out with tap water to remove adhering soil particles and debris. Then they were put in 250 mL Schott flasks filled with 20% EtOH and stored in the dark at 6°C till they were scanned yielding digital black and white images (600 dpi) which were used for determining morphological root traits including total root length (TRL), root surface area (RSA) and the average root diameter (ARD), by the means of WinRhizo® Pro. (Regent Instruments Inc., Quebec, Canada) according to the protocol developed by Himmelbauer (2004). Finally, they were oven-dried at 65°C for 72 hours to determine the root DM. Later on the specific root length (SRL), which has been defined as the ratio of root length to root dry matter, has been also calculated.

### 3.6. Analysis of shoot and seed phosphorus content

In order to determine the seed P content as well as the shoot P uptake, P concentrations of the seeds, leaves and stems were determined separately in mg P $g^{-1}$ sample. Oven-dried seed, leaf and stem samples were ground separately using a RETSCH® ball mill (Schwingmühle MM 400) for typical grinding time of 14 seconds with vibrational frequency of 20 Hz providing a homogenous powder with $d_{90}$ < 5 µm final fineness (according to the device specifications contained in the

manual). The ground samples were emptied out into PCR tubes (Eppendorf® Reaktionsgefäß 5 ml) and they were dried by heating them in the oven at 105 °C for about 4 hours and cooled in a desiccator. They were subsequently weighed with Sartorius® BP61 lab-scale for 100 ± 1 mg. Then the ground samples were transferred to CEM-Xpress Teflon vessels and subsequently submerged in 4 mL $HNO_3$ overnight. On the next day 1 mL of $H_2O_2$ 30% w/v (300 g $H_2O_2$ per 1000 mL total volume distilled water) was poured into each vessel, then the samples were fully digested by acid cooking in a "MARS 6 System" microwave (CEM GmbH) at 1030-1080 W for 20 minutes (generates about 200°C). The digests were filtered through P-free filter papers (Macherey-Nagel GmbH & Co. KG) into 25 mL flasks and topped up with demineralized water. The digests were later diluted with demineralized water for the measurements by ICP-OES (Perkin-Elmer Optima 3300 DV).

### 3.7. Statistical analysis

After checking the normality with Kolmogorov–Smirnov procedure and the homogeneity of variances with Levene's test, general linear models for one or two-way ANOVA were calculated using IBM® SPSS® Statistics version 21. Significant differences of means were determined by using Tukey's HSD mean separation test ($P \leq 0.05$) in EXP1; also in case of a significant interaction in EXP2 for LP and HP data separately. To test for effects of seed P content on above-ground biomass production and shoot P uptake in the early growth period (prior to anthesis), the relationships of seed weight and seed P with shoot biomass and shoot P uptake were determined using bivariate linear regression models using the statistical software SigmaPlot™ version 14.0. Linear regressions were also calculated for relationships between other traits of interest.

## 4. Results and Discussion

### 4.1. Results of EXP1

One-way ANOVA showed a significant difference among genotypes at $p < 0.01$ for seed dry matter (DM), seed P content, leaf, stem, shoot, root and total plant DM, leaf area, shoot:root ratio, leaf P concentration, stem P concentration, shoot P uptake, TRL, SRL and at $p < 0.05$ for seed P concentration and RSA. The ARD values were, however, not significantly different (Table 6).

Table 6. Results of one-way ANOVA examining the effects of genotype in EXP1.

| Independent variable | P values for Genotype effect |
|---|---|
| Seed DM | <0.01 |
| Seed P content | <0.01 |
| Seed P concentration | <0.05 |
| Leaf DM | <0.01 |
| Stem DM | <0.01 |
| Shoot DM | <0.01 |
| Root DM | <0.01 |
| Total plant DM | <0.01 |
| Shoot:Root ratio | <0.01 |
| Leaf area | <0.01 |
| Stem P concentration | <0.01 |
| Leaf P concentration | <0.01 |
| Shoot P uptake | <0.01 |
| Total root length (TRL) | <0.01 |
| Root surface area (RSA) | <0.05 |
| Average root diameter (ARD) | n.s. |
| Specific root length (SRL) | <0.01 |

n.s.: not significant

As presented in Fig. 1, the tested soybean genotypes differed in seed weight i.e. seed DM (Fig. 1a), seed P content differs too (Fig. 1c) and it is closely (linearly) related to seed weight (Fig. 1d). Seed P concentration is also significantly partially variable in that set of soybean genotypes (Fig. 1b). The maximum and minimum seed P concentrations, which have been measured for the varieties in this experiment, were 5.76 mg $g^{-1}$ and 6.79 mg $g^{-1}$, respectively (Fig. 1b).

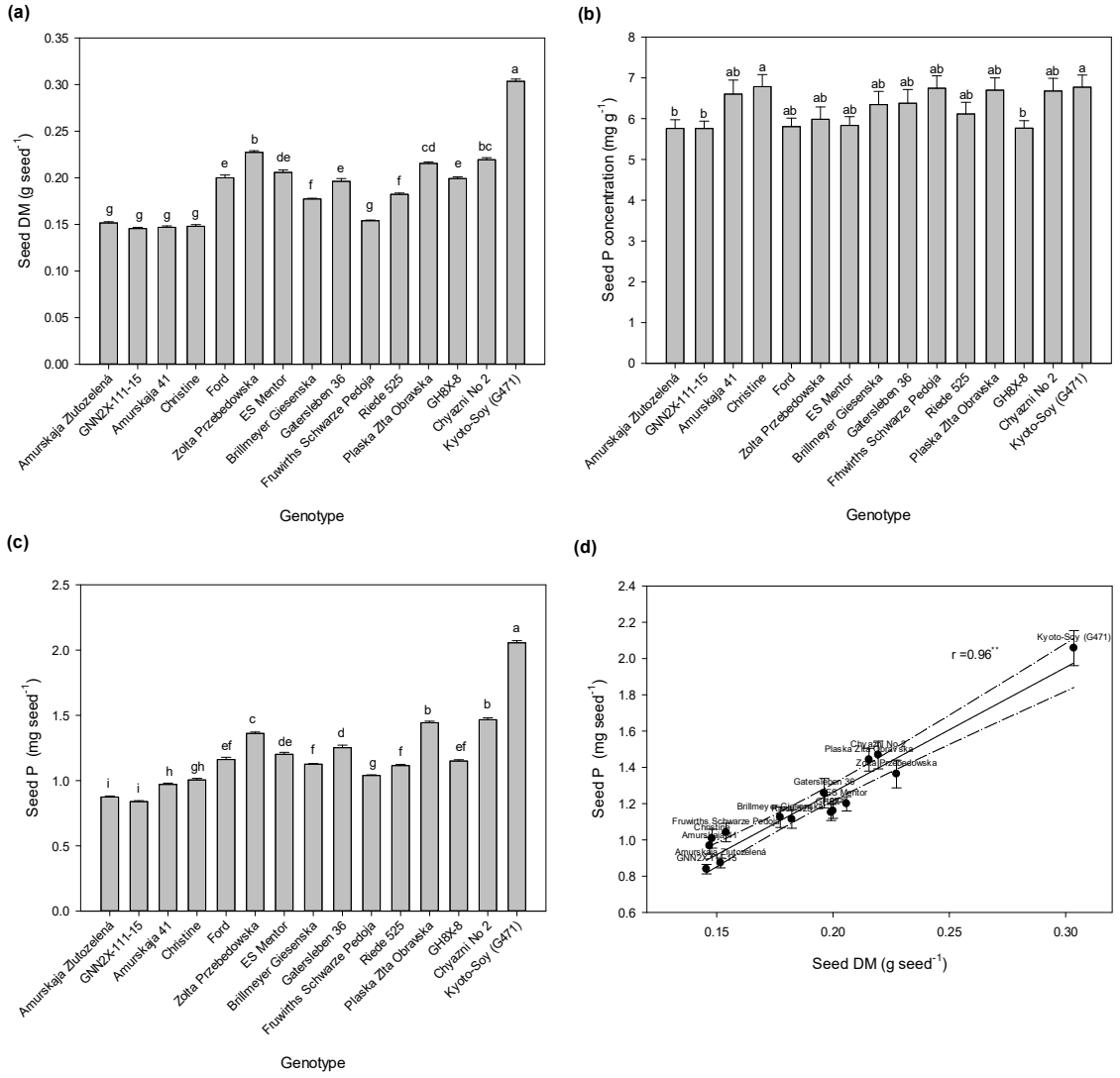


Fig. 1. Seed DM (a), seed P concentration (b) and seed P content (c) among the soybean genotypes. The letters on top of each column represent Tukey's HSD grouping. Error bars represent standard errors. The linear relationship between seed P content and seed DM is also shown (d).

Multiple comparisons among means also show significant differences between specific genotypes, most importantly in total amount of aboveground biomass and shoot P uptake (Fig. 2). It has been observed that the genotype Kyoto Soy was the most productive genotype by far in comparison to all other genotypes with regard to shoot DM and shoot P uptake.

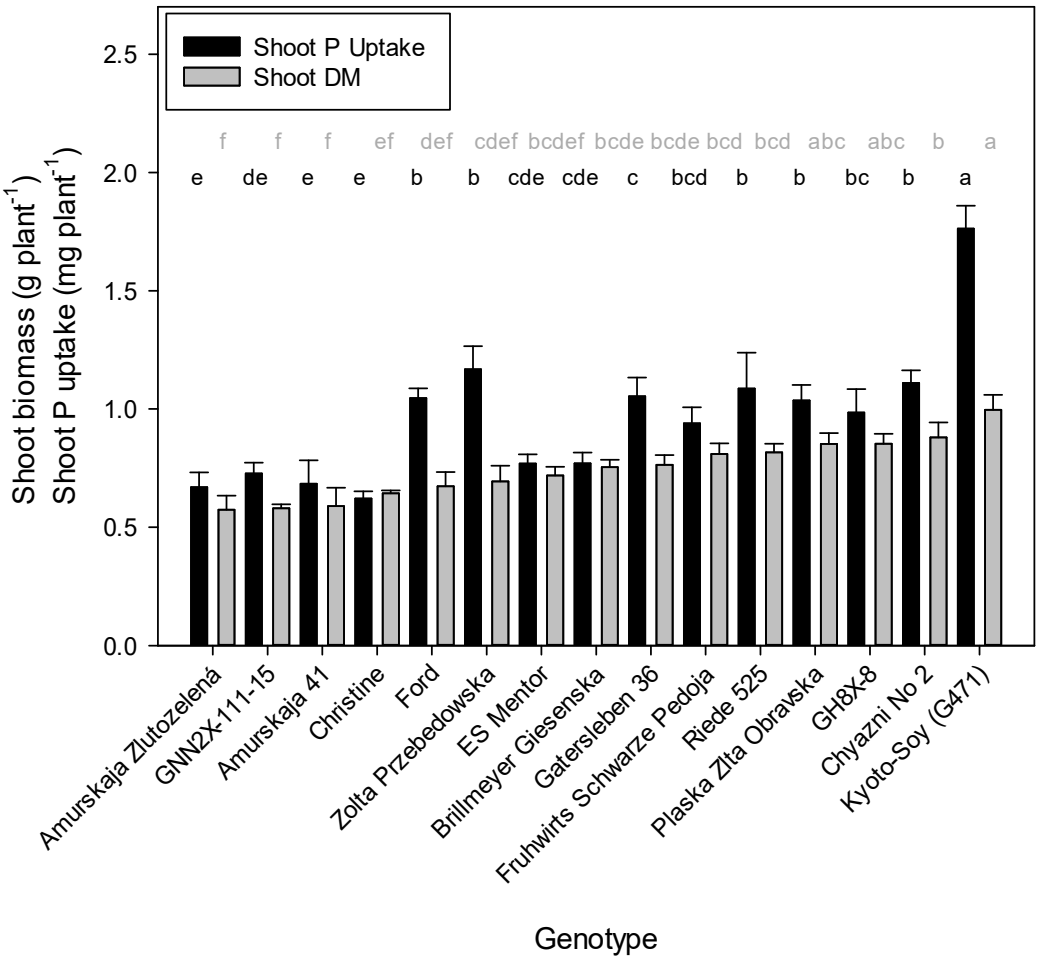


Fig. 2. Shoot P uptake and shoot biomass at 30 DAP, sorted for the shoot DM. The letters on top of each column represent Tukey's HSD grouping. Error bars represent standard errors.

As it can be seen in Fig. 2, where the genotypes are sorted for their shoot DM in ascending order, within a range of biomass values that are not statistically different, we have had significantly different shoot P uptake values. For instance, Christine, Ford, Zolta Przebedowska and ES Mentor were not statistically different regarding their shoot DM value; nevertheless, Ford and Zolta Przebedowska have accumulated more P in the shoots in comparison to Christine and ES Mentor, which indicates a high shoot P concentration for these two genotypes. A comparatively high shoot P concentration was also recorded for Kyoto-Soy while Christine and Brillmeyer Giesenska had the lowest P concentration in the aboveground organs (Fig. 3). Therefore, in Kyoto-Soy not only the biomass, but also the shoot P concentration was the highest.

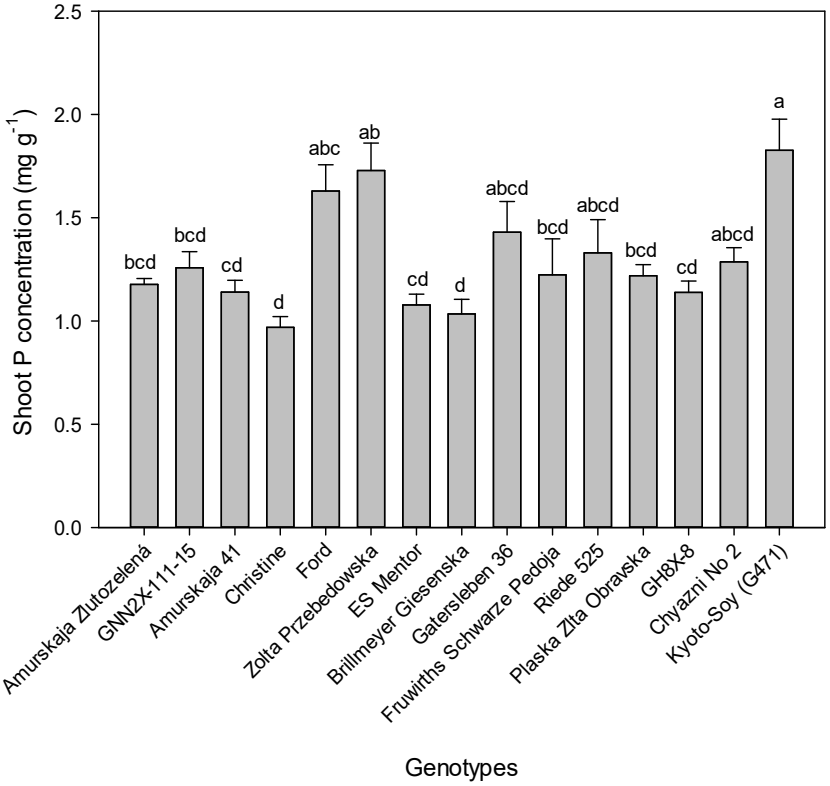


Fig. 3. Shoot P concentration. The letters on top of each bar represent Tukey's HSD grouping. Error bars represent standard errors.

Consequently, it has been shown that shoot DM and also total DM of plants at 30 DAP were different between genotypes and closely (linearly) related to seed DM as well as seed P content (Fig. 4a & 4c, also 4d & 4f). On the contrary, there was no significant correlation between root DM and seed size or seed P content (Fig. 4b & 4e).

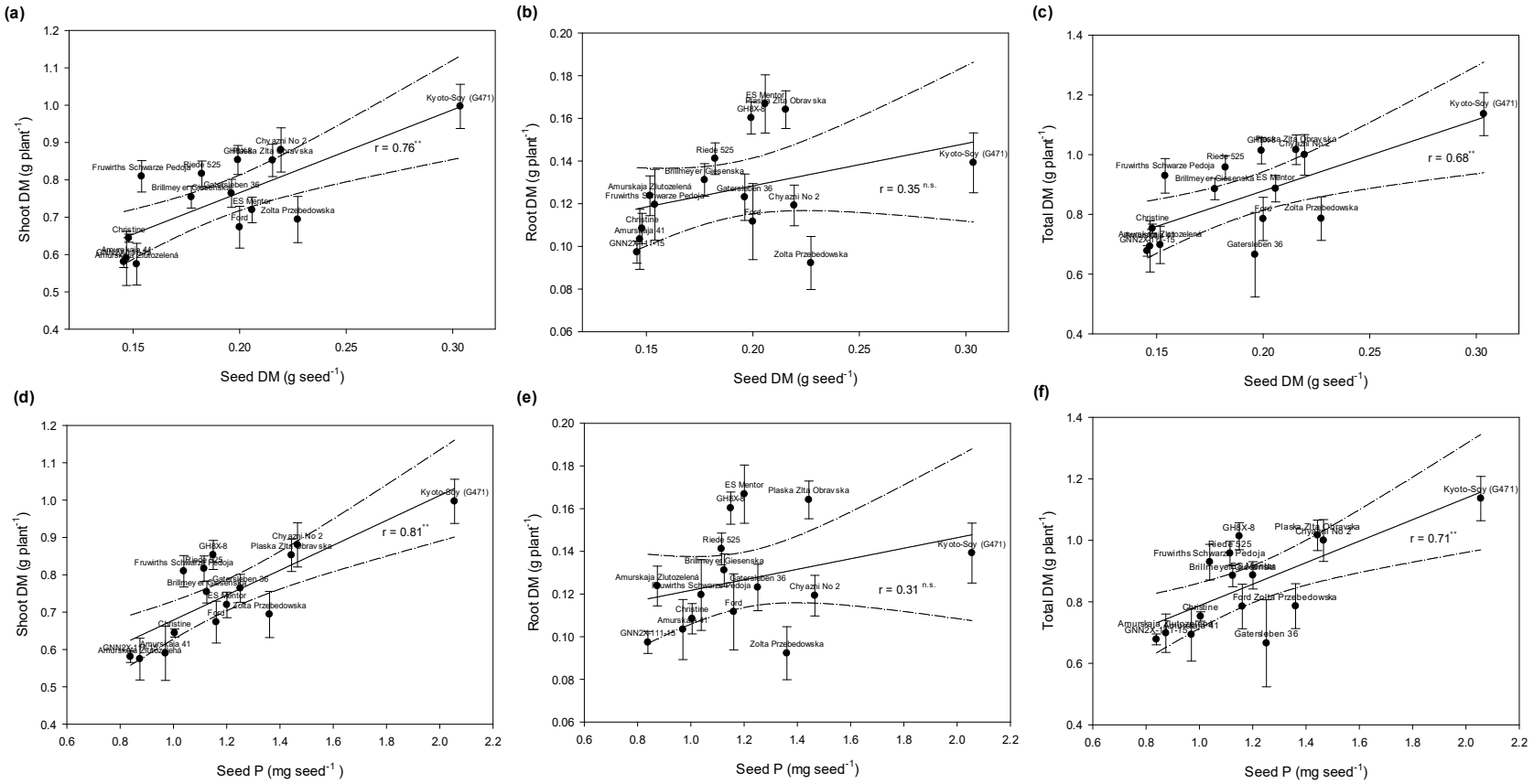


Fig. 4 Correlations of seed DM and seed P with shoot, root and total plant DM. Error bars represent standard errors.

Furthermore, as shown in Fig. 5, the shoot P content of plants 30 DAP is closely linearly related to seed P content ($r=0.9079$, $p < 0.05$). The absolute amount of shoot P content is a linear function of seed P content with an intercept close to 0 ($y_0=-0.23$) and a slope slightly less than 1 ($a=0.97$), reflecting the root P content and residual P in seed tissue (Fig. 5).

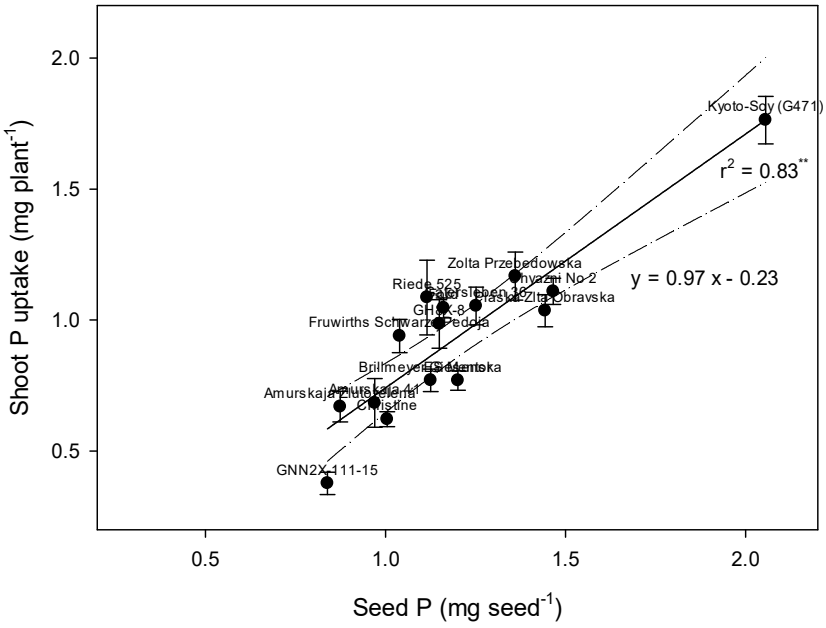


Fig. 5. Linear regression of shoot P content on seed P content. Error bars represent standard errors.

Obviously there is a pattern which has been repeated in seed DM, seed P content and finally shoot P uptake variations (cf. Fig. 1, 2; all graphs are sorted in ascending order for shoot biomass to make the comparison easier). So it can be argued that the higher seed P contents which arise from bigger seeds in soybean are accountable for higher shoot P uptake under low P supply.

There are different approaches to assess the significance of the role that seed P plays in supplying shoot P. The best, but most demanding scenario will be to study the phosphorus dynamics from seed to the plant very much in detail. This requires the measurement of P in the root as well as in cotyledons and seed coat after they are fully used. Since the overall mean for root DM was about 127 mg, it was not feasible to grind it and analyze the P using the microwave-assisted digestion method. Therefore, it has been tried to focus on the proportion of the seed P and shoot P and their

correlation. Fig. 6 shows the contribution of the seed P content to the P nutrition of the shoots. This ratio for different genotypes was between 61.9% and 97.4% with hardly any significant differences between 15 genotypes. The average value was 79.7%, meaning that the seed P reserves alone contributed very much to the P supply of the aboveground biomass until 30 DAP.

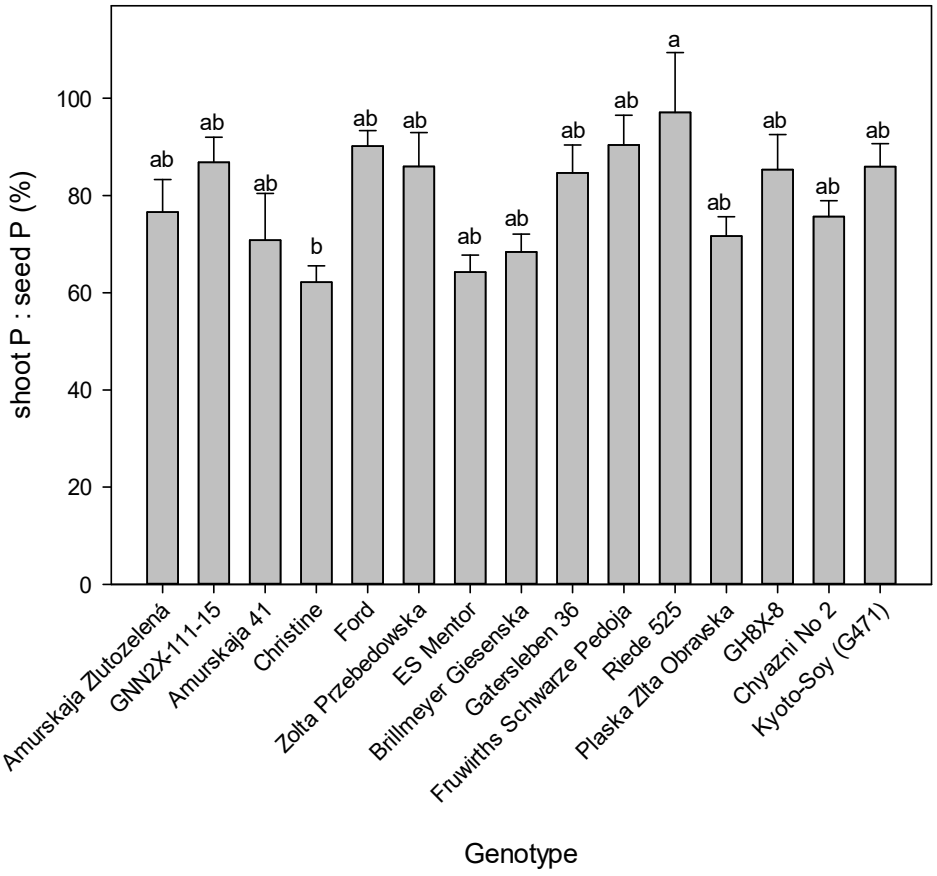


Fig. 6. Ratio of shoot P to seed P in percentage. The letters on top of each column represent Tukey's HSD grouping. Error bars represent standard errors.

Despite the significant differences for seed P contributions to shoot P according to the results of ANOVA test ($p<0.05$), most of the genotypes had similar mean values for the mentioned ratio. Only in cv. Riede the role of seed P in supplying the shoot P was significantly higher than in cv. Christine. The differences in shoot P:seed P ratio can be explained by the differences in the shoot P uptake and also by the differences in the seed DM and consequently seed P content. Here Christine, which showed the lowest mean value in the shoot P:seed P ratio, had also the lowest

shoot P uptake among all genotypes (77.49% lower than the mean value) (Fig. 7). On the other hand, Riede despite its relatively small seeds and regardless of its seed P content, which was 7.67% lower than overall seed P content, had taken up 25.77% more P in the shoot when compared to the overall mean value. The high shoot P: seed P ratio of nearly 99% might also indicate the acquisition of some soil P by Riede.

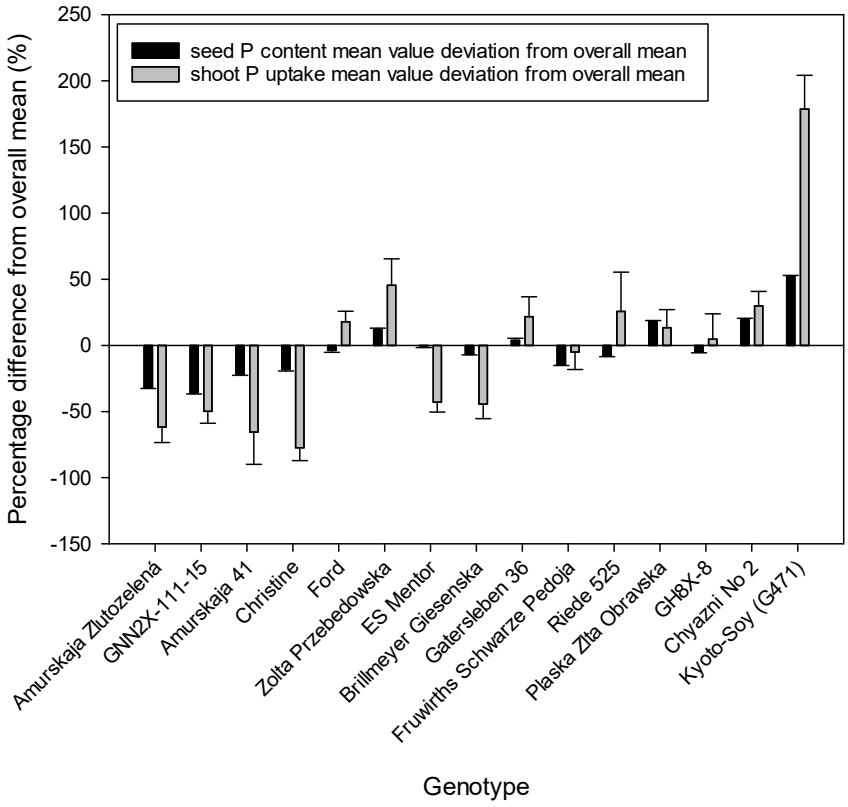


Fig. 7. Shoot P and seed P content deviation from overall mean values.

One has also to note that some of the seed P may have been also invested in the roots, which could have indirectly resulted in higher shoot P due to an earlier and better root system establishment. This question can be partly answered by looking at the shoot:root allometry. If the shoot:root ratio is different, so is presumably the compartmentation of P inside the soybean plants. As shown in Fig. 8, the difference in shoot:root ratio between some of the varieties was substantial in such a way that Zolta and Fruwirths have higher shoot:root ratio compared with Amurskaja, ES Mentor, Plaska and GH8X-8. One example is Zolta Przebedowska. Despite its lower than average shoot biomass, Zolta showed the highest shoot:root ratio, meaning that more assimilates and metabolites, presumably including more P, are allocated to the shoot.

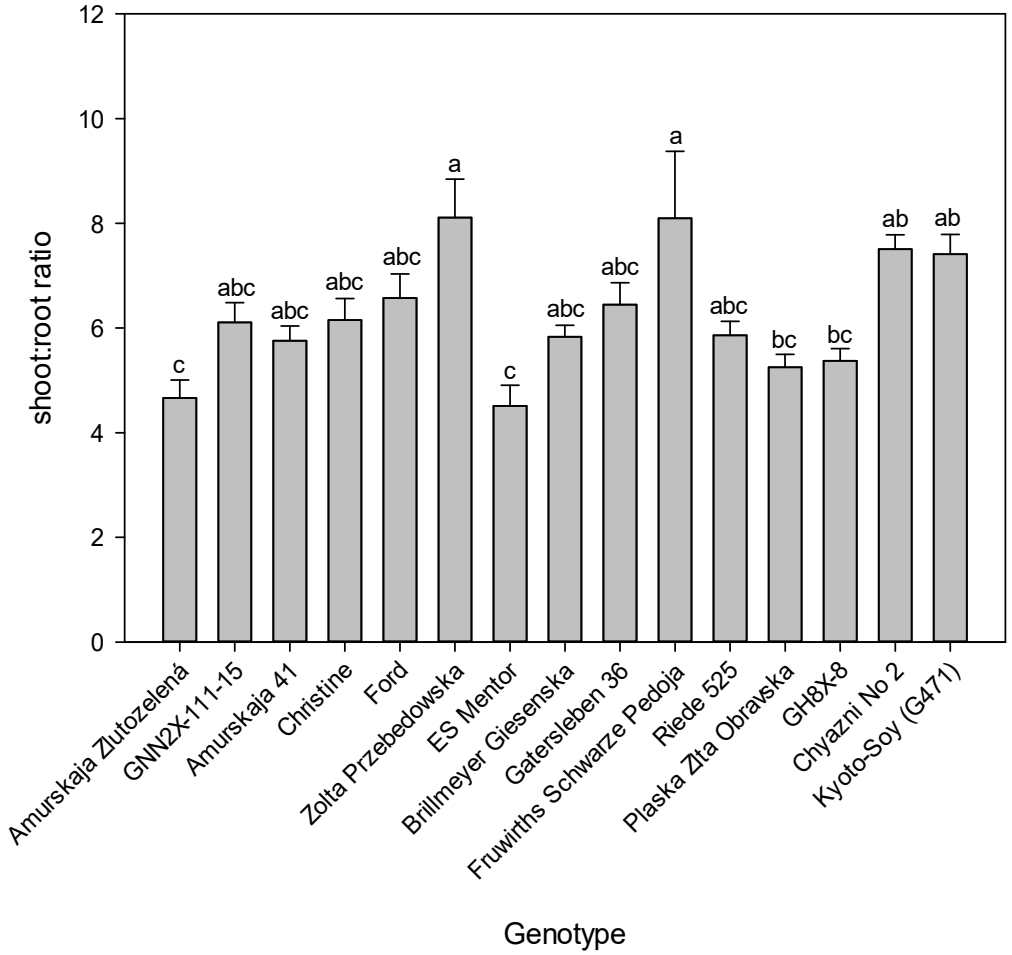


Fig. 8. Shoot:root allometry of the soybean genotypes. The letters on top of each column represent Tukey's HSD grouping. Error bars represent standard errors.

Furthermore, the difference between the P content in the shoot and the seed P content is presented in Fig. 9. This shows the absolute amount of P in seeds that has not arrived in the shoots, assuming that the whole P in the shoot is supplied by the seed P reservoir and no P has been supplied by roots from the substrate. Due to its low level of phyto-available P this seems plausible. The absolute difference shows in comparison with the relative values (cf. Fig. 6) that besides Christine, ES Mentor and Plaska Zlta Obravska also with Gatersleben comparatively much seed P was not transferred to the shoot. Gatersleben was a genotype with values for shoot P and seed P which were both above the overall mean, but Gatersleben accumulated almost 10% more P in shoots than the overall mean, also due to a relatively high shoot P concentration (cf. Fig. 3), while its seed P content was only 4% higher than the average seed P content.

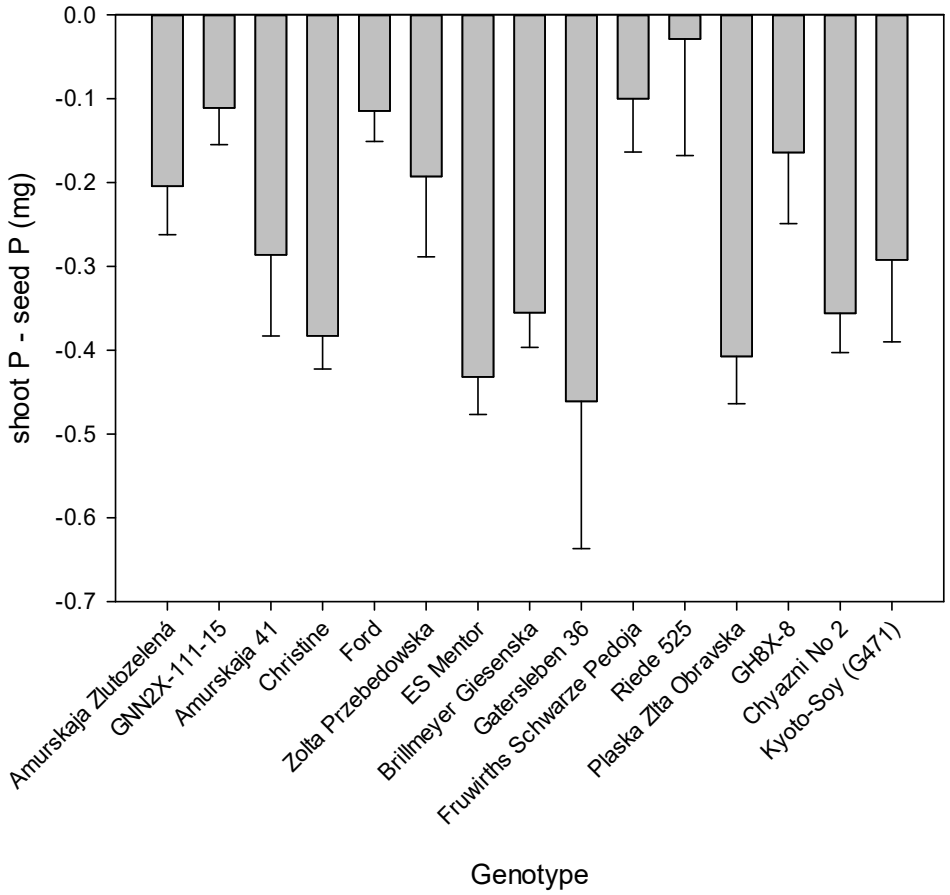


Fig. 9. Differences between shoot P uptake and seed P content. Error bars represent standard errors and Tukey's HSD test ($P < 0.05$) revealed no pairwise differences between the genotypes.

With regard to the studied root traits, we found differences between genotypes in root DM and several root traits (i.e. TRL, RSA and SRL), but they do not contribute significantly to explain the differences in shoot P content, approving that root P uptake during that early phase of plant growth plays no important role. In detail, root DM, as a substantial factor controlling the root P content, does not significantly correlate with seed size and seed P content (Fig. 4b & 4e). Also the correlations of root DM (Fig. 10), TRL (Fig. 11), RSA (Fig. 12) and SRL (Fig. 13) with the acquired P in the shoot were positive, yet not significant, indicating that producing more roots under low P phyto-availability does not necessarily enhance the shoot P uptake at 30 DAP.

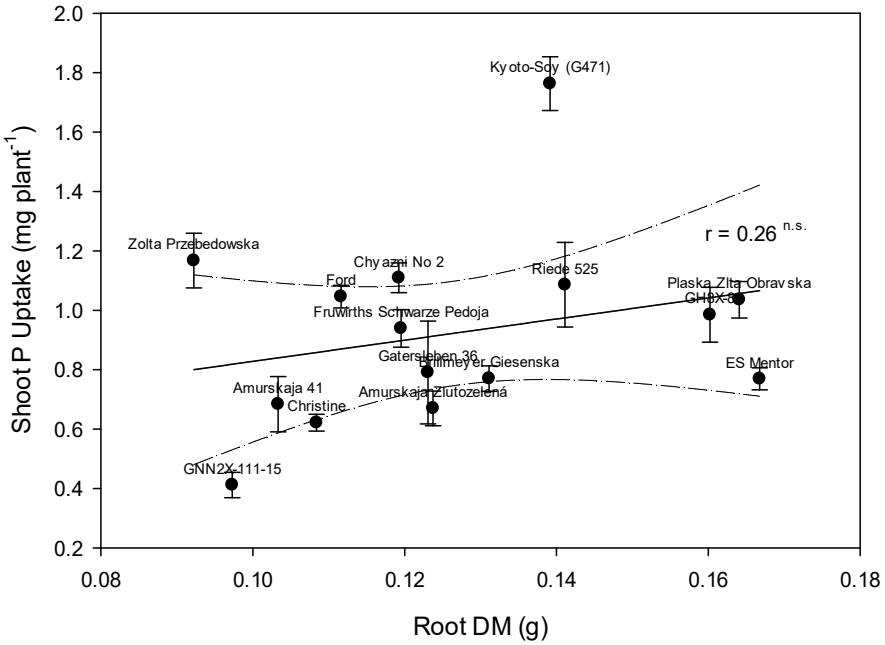


Fig. 10. Correlations of root DM with shoot P uptake. Error bars represent standard errors.

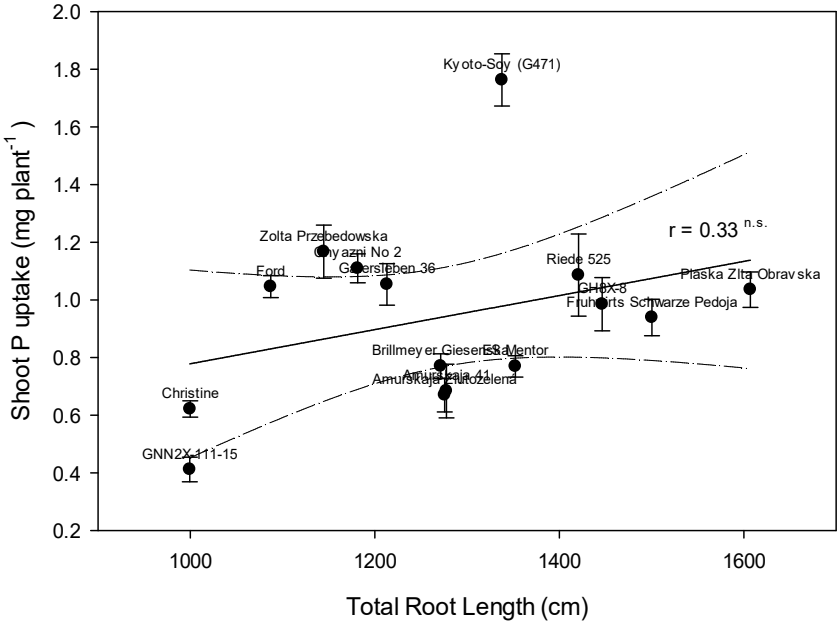


Fig. 11. Correlations of TRL with shoot P uptake. Error bars represent standard errors.

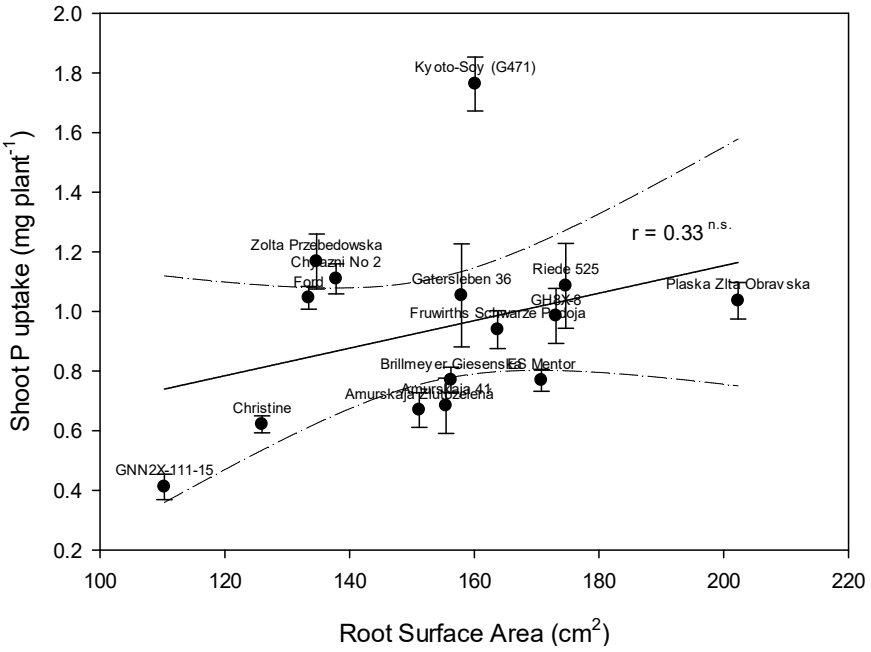


Fig. 12. Correlations of RSA with shoot P uptake. Error bars represent standard errors.

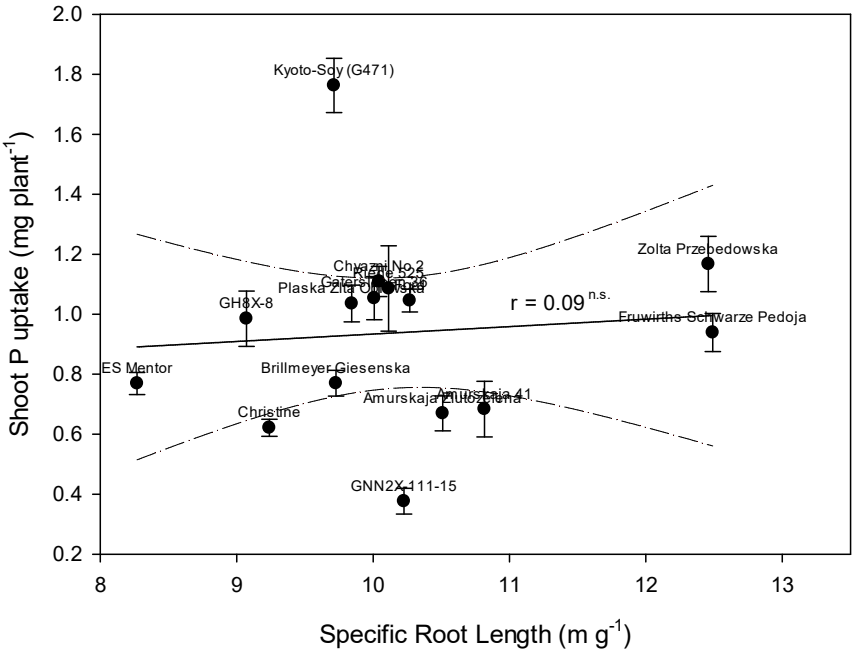


Fig. 13. Correlations of SRL with shoot P uptake. Error bars represent standard errors.

4.2. Results of EXP2

As far as the effects of genotypic variations and P supply are concerned, two-way ANOVA showed a significant genotype effect for shoot DM and its components (stem DM and leaf DM) as well as root DM, total plant DM, shoot:root ratio, stem P concentration, leaf P concentration, shoot P concentration, shoot P uptake, total root length (TRL), average root diameter (ARD), root surface area (RSA), specific root length (SRL) and shoot P:seed P ratio at 40 DAP (Table 7). The effect of P supply was also significant for all aforementioned variables except for SRL. The interaction of main effects was significant for all variables except for the interactions on stem, leaf and shoot P concentrations, shoot P uptake, TRL and RSA. The earliest morphological differences indicating the effect of P supply on the growth and development of all five genotypes at 25 DAP are shown in Fig. 14.

Table 7. Results of one and two-way ANOVA examining the effects of genotypes and P supply levels in EXP2.

| Independent variable | P Values | | |
|---|---|---|---|
| | Genotype | P supply | Genotype x P supply |
| Seed Characteristics [1] | | | |
| Seed DM | <0.01 | n.a. | n.a. |
| Seed P content | ≤0.01 | n.a. | n.a. |
| Seed P concentration | ≤0.05 | n.a. | n.a. |
| Difference of seed P content from overall mean (%) | ≤0.001 | n.a. | n.a. |
| Dry Biomass [2] | | | |
| Leaf DM | ≤0.001 | ≤0.001 | ≤0.01 |
| Stem DM | ≤0.001 | ≤0.001 | ≤0.01 |
| Shoot DM | ≤0.001 | ≤0.001 | ≤0.001 |
| Root DM | ≤0.001 | ≤0.001 | ≤0.001 |
| Total Plant DM | ≤0.001 | ≤0.001 | ≤0.001 |
| Shoot:Root ratio (%) [2] | ≤0.001 | ≤0.001 | ≤0.001 |
| Stem P concentration [2] | ≤0.05 | ≤0.01 | n.s. |
| Leaf P concentration [2] | ≤0.001 | ≤0.05 | n.s. |
| Shoot P uptake [2] | ≤0.001 | ≤0.05 | n.s. |
| Shoot P concentration[2] | ≤0.001 | ≤0.05 | n.s. |
| Total root length (TRL) | ≤0.001 | ≤0.05 | n.s. |
| Average Root Diameter (ARD) [2] | ≤0.05 | ≤0.01 | ≤0.01 |
| Root Surface Area (RSA) [2] | ≤0.05 | ≤0.001 | n.s. |
| Specific Root Length (SRL) [2] | ≤0.001 | n.s. | ≤0.01 |
| Shoot P:Seed P (%) [2] | ≤0.001 | ≤0.001 | ≤0.001 |

[1] tested using a one-way ANOVA with genotype as factor. [2] tested using a two-way ANOVA with genotype and P supply as factors.

n.a.: not applicable

n.s.: not significant


Fig. 14. The first appearance of morphological differences indicating the effect of P supply on the growth and development of all five genotypes at 25 DAP. Genotypes from left to right: Zolta Przebedowska, Riede 525, Ford, ES Mentor and Kyoto-Soy (G471)

As presented in Fig. 15, the 5 soybean genotypes which were studied in this experiment showed significant variations in seed size, i. e. seed DM (Fig. 15a), seed P content differed too (Fig. 15c) and it is closely (linearly) associated with the seed DM (Fig. 15d). Also, the seed P concentration is significantly different in that set of soybean genotypes with Kyoto revealing a higher seed P concentration than all the others (Fig. 15b). Seed weight was strongly correlated with seed P content for this experiments ($r = 0.98$, $p \leq 0.05$). The maximum and minimum seed P concentrations, which have been measured for the varieties in this experiment, were 5.76 mg $g^{-1}$ and 6.79 mg $g^{-1}$, respectively.

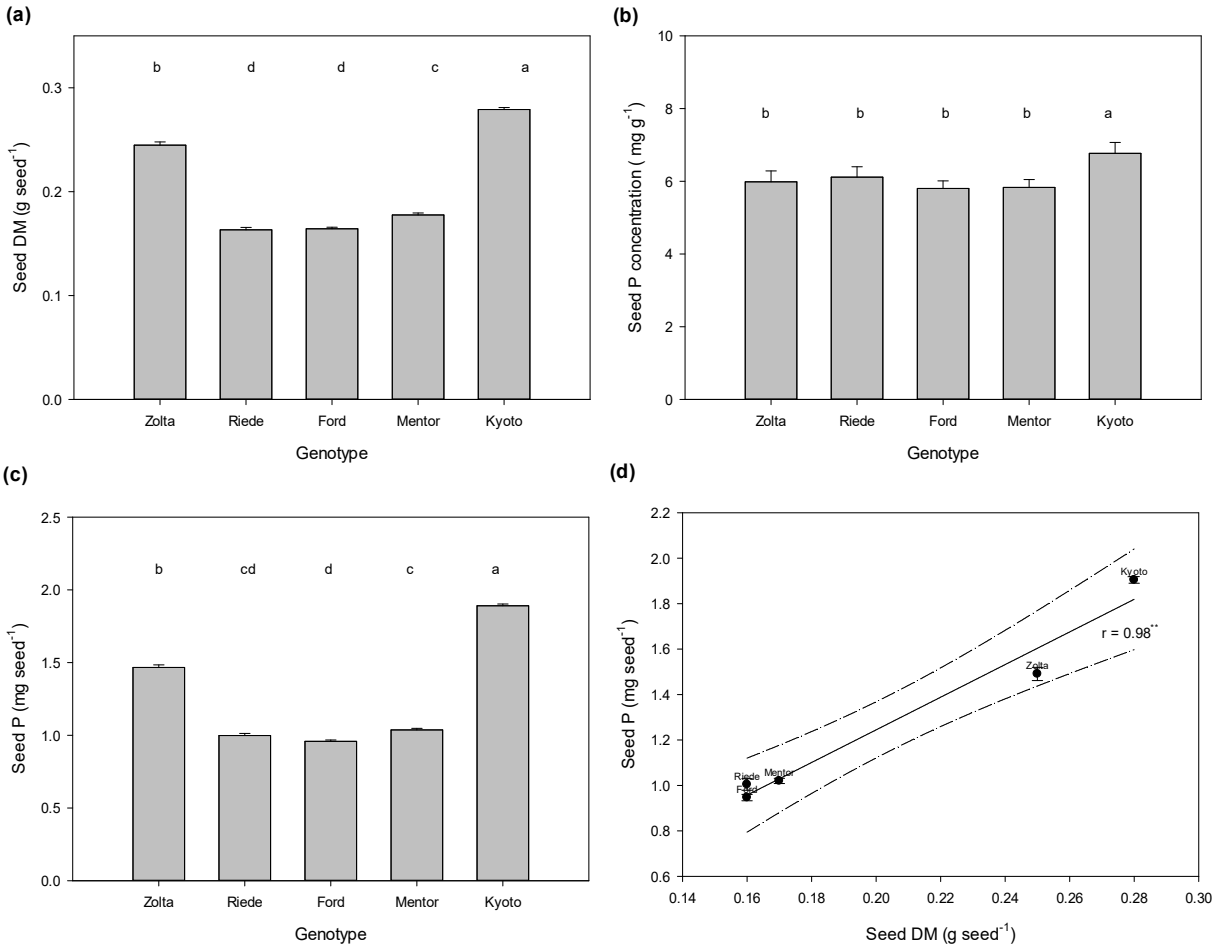


Fig. 15. Seed DM (a), seed P concentration (b) and seed P content (c), also the linear relationship between seed P content and seed DM among the soybean genotypes (d). The letters on top of each column represent Tukey's HSD grouping. Error bars represent standard errors.

Shoot DM was affected by the interaction of genotype and P supply, single pairwise comparisons between the means under LP and HP separately using Tukey HSD show that Zolta and Kyoto were more productive at LP, whilst at HP Riede and Kyoto were more productive when compared to least productive genotype Ford (Fig.16a). Nevertheless, a similar pattern has been repeated at both LP and HP between the genotypes regarding shoot DM, except for genotype Riede at HP which showed a better response to P application by accumulating more biomass in comparison to other genotypes. A pattern similar to the shoot DM pattern, also applies to genotypic variations in shoot P uptake and Riede, despite the higher shoot DM, also falls within that trend, suggesting that the higher level of biomass accumulation in Riede at HP was at the cost of P dilution in the

shoot tissues (Fig. 17). The opposite of dilution effect, i.e. increased P concentration at HP, can be seen in Ford, where less biomass accumulation resulted in significantly higher concentration of P, when compared to Riede (also Fig. 17). However, the variations in the shoot P components (shoot DM and shoot P concentration) in Riede and Ford at HP compensate each other and therefore the trend of shoot P uptake of all genotypes at HP and LP was similar.

Also as shown in Table 7, there was no significant interaction of P supply and genotype regarding shoot P uptake, and therefore the effect of genotypes on shoot P uptake is not different at different levels of P supply. But while shoot P uptake differs hardly between the genotypes at LP in absolute figures, the genotypic variation for shoot P uptake at HP shows larger differences. Here Ford and Kyoto showed the lowest and highest shoot P uptake, meaning that Kyoto and somehow Zolta were more responsive to P supply in terms of P uptake when compared to Ford or Mentor. Both, Kyoto and Zolta, showed also a better performance regarding P acquisition in the shoot at LP. This could in reference to Rose *et al*. (2013) be interpreted as low P-tolerant or in reference to Manschadi *et al*. (2014) as P-efficient. The results also imply that at LP, where the shoot P concentrations were similar, shoot DM played the main role in determination of the shoot P uptake, while at HP differences in shoot P concentrations become more important.

Biomass accumulation in the roots of soybeans is shown in Fig. 16b. The interaction of genotypic and P supply effects affected the belowground biomass accumulation significantly. The trend of genotypic variations of root DM has similarities to the shoot DM variations. Just like with the shoot DM. also here the trends of genotypic variations of root DM at LP and HP are different. Considerable variation in root DM can be observed at HP, whilst at LP these variations were not significant. Like the case of shoot DM at HP, the highest and lowest root DM accumulation at HP belongs to Riede (also Mentor) and Ford, respectively. Specifically genotype Riede, with its high root DM accumulation at HP, is mainly responsible for the interaction between the factors. The genotypes Riede, Mentor and Ford are revealing contrasting root DM, which suggests differences in potential use of exogenous P from the soil in the forthcoming stages of plant growth due to root system size or just increased metabolic costs for soil exploration.

As presented in Fig. 16c, shoot:root ratio declined in plants grown in P limited substrate. Larger shoot:root ratios at HP are due to a relatively greater increase in shoot growth than in root growth with P application. The absolute and relative gap of root DM between LP and HP is smaller than the same gap for shoot DM. On average, the soybean genotypes at HP accumulated 2.99 folds more biomass in the shoots when compared to LP, while the increase in root DM due to high P supply was only 1.83 folds. Lowest shoot:root ratio was observed in ES Mentor at both HP

(statistically different to Ford and Kyoto) and LP treatments (statistically different to Zolta and Kyoto). The highest shoot:root ratio under HP occurred in Ford and Kyoto while under LP Kyoto had a higher shoot:root ratio compared to all other genotypes.

Total DM of plants, which is the sum of root and shoot DM 40 DAP (Fig. 16d), were also different between the two P supply levels and were genotypically following the same trend of variations as shoot DM with identical statistical groupings. The root DM was much less when compared to shoot DM, which makes its effect on total plant DM close to negligible. Regarding the interaction of genotype and P supply, it is again the high value of Riede at HP which makes the interaction of factors significant.

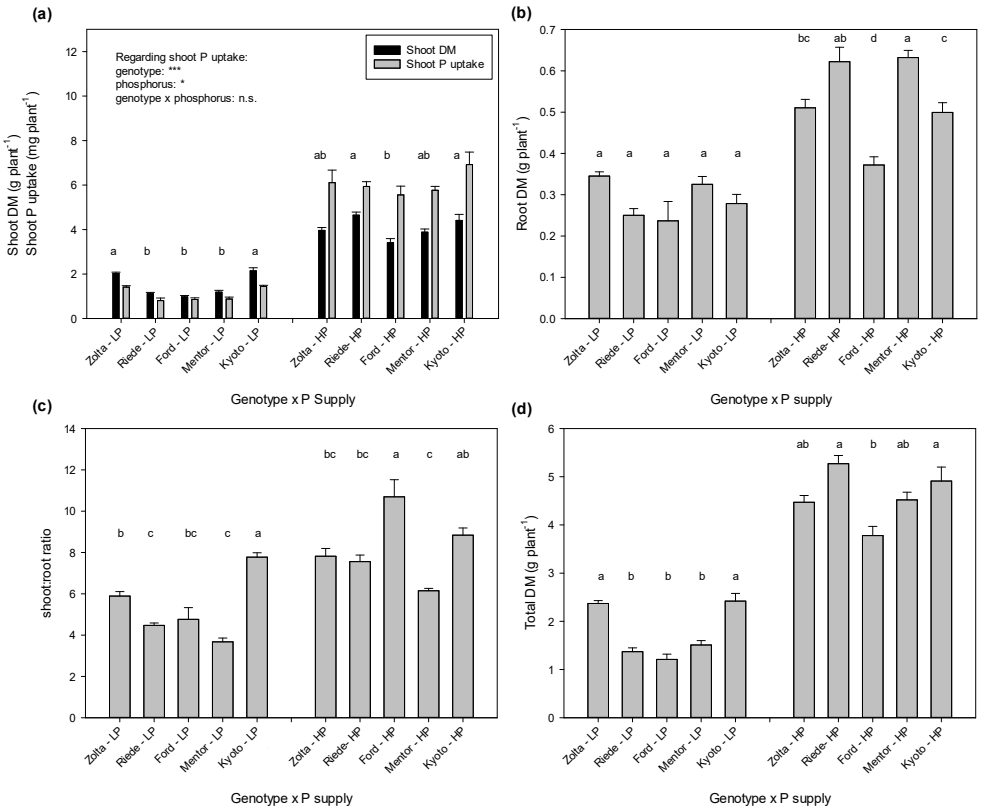


Fig. 16. Shoot DM and P uptake (a), Root DM (b), shoot:root ratio (c) and total plant DM (d), for soybean genotypes at LP and HP. The letters on top of each column represent Tukey's HSD grouping. Error bars represent standard errors.

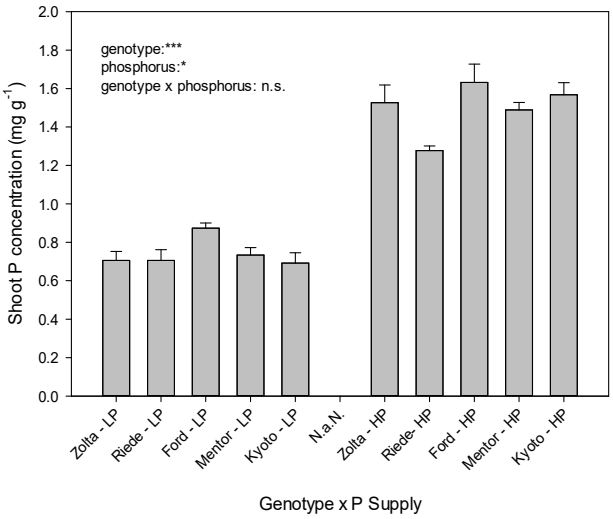


Fig. 17. Shoot P concentration for soybean genotypes at LP and HP. Error bars represent standard errors.

As shown in Fig. 18, in spite of the strong positive correlation between seed size with shoot DM (Fig. 18a) and total plant DM (Fig. 18c) at LP (r=0.99 and r=0.98, respectively), the strength of the relationship of the same variables at HP was weak and non-significant. Similar strong positive correlations between seed P content with shoot DM (Fig. 18d) and total plant DM (Fig. 18f) at LP are also recorded (r=0.96 and r=0.94, respectively), whilst the strength of the relationship of the same variables at HP was again weak and non-significant. The dependencies of root DM on seed size and seed P content were not significant at both LP and HP treatments as shown in Fig. 18b and Fig. 18e.

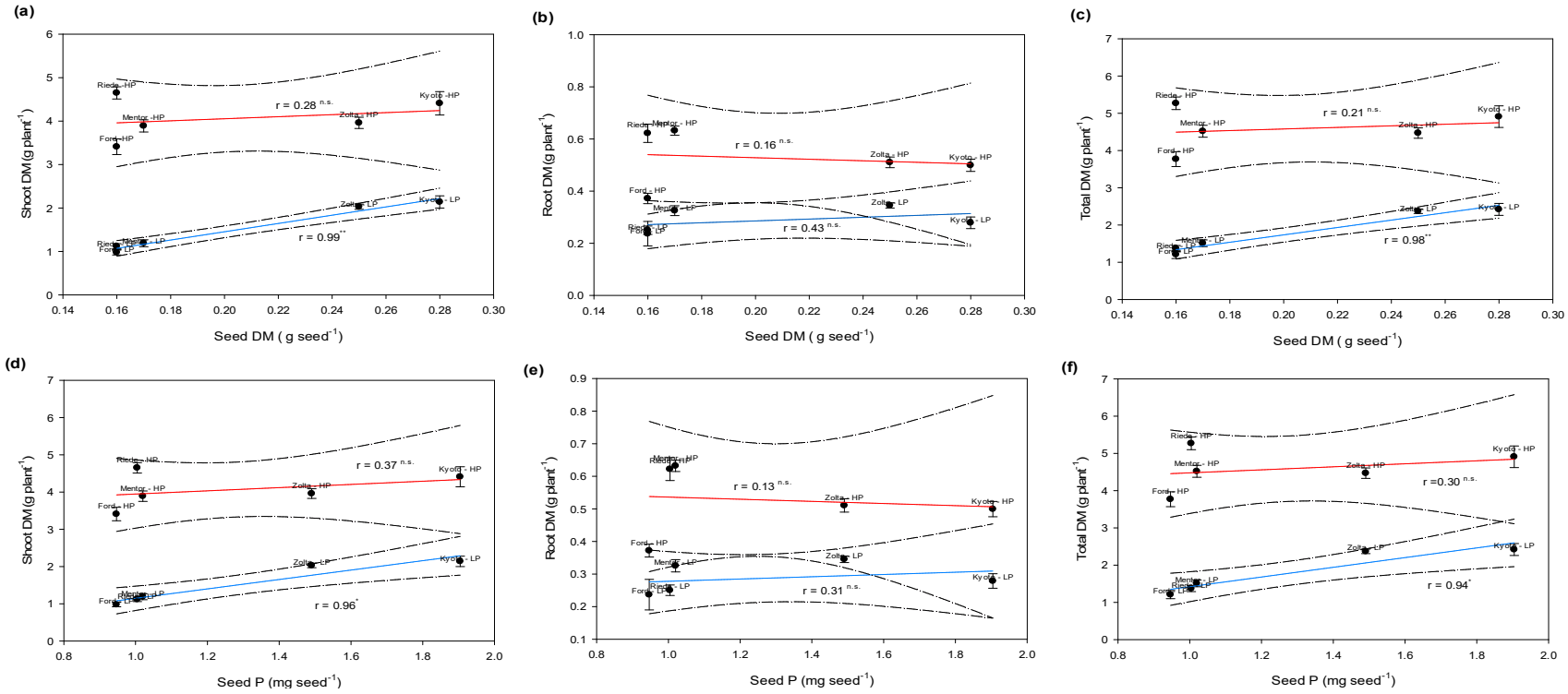


Fig. 18. Correlations of seed DM and seed P content with shoot DM (a & d), root DM (b & e) and total plant DM (c & f). Error bars represent standard errors.

We have estimated linear regressions between seed size or rather seed P and shoot P to estimate the significance and the function parameters (intercept and slope). In case of correlation between seed P and shoot P (Fig. 19b), we found that the slope for each P supply level was significant (a= 1.19 and 0.72 for HP and LP, respectively). Obviously the regression slope at HP was 1.48 time higher than at LP. Additionally, it was below or above 1 for HP or LP, respectively. The dependency of shoot P on seed weight was also significant for both P levels (Fig. 19a). It is evident that at both HP and LP, the shoot P content is highly positively correlated with seed DM ($r = 0.88$ and $r = 0.99$, respectively). Also here the regression slope at HP is 1.7 times higher than that for LP. This underlines the importance of seed size for shoot growth and P uptake.

As presented in Fig. 20, neither seed size nor seed P are significantly correlated with TRL at both LP and HP. The negative slopes of the regression lines in Fig. 20, although not significant, may indicate a mild negative relationship of seed DM or seed P with TRL.

The contribution of seed P to shoot P uptake can be expressed in different ways. It can be discussed as the ratio (shoot P:seed P), as used by Assefa *et al.* (2018) for similar traits, or as difference (shoot P minus seed P) (Fig. 21-22).

One way is the evaluation of shoot P:seed P in the form of percentage as it is presented in Fig. 21. Two-way ANOVA had revealed a significant difference in the average shoot P:seed P ratio among the genotypes of soybean ($p \leq 0.001$), the P supply levels ($p \leq 0.001$) and also their interaction ($p \leq 0.001$). The average shoot P:seed P ratios at LP and HP were respectively 86% and 503%. At LP the ratio shoot P:seed P was close to 1:1, underlining the importance of seed P in fulfilling the P requirements of the young soybean plants under P starvation. Comparison of means revealed that the mentioned proportions for all genotypes at LP were not statistically different whilst variation exists among genotypes at the HP treatment. Here Riede and Ford showed much larger shoot P:seed P ratios when compared to Zolta and Kyoto, and Mentor also had a higher proportion than Kyoto.

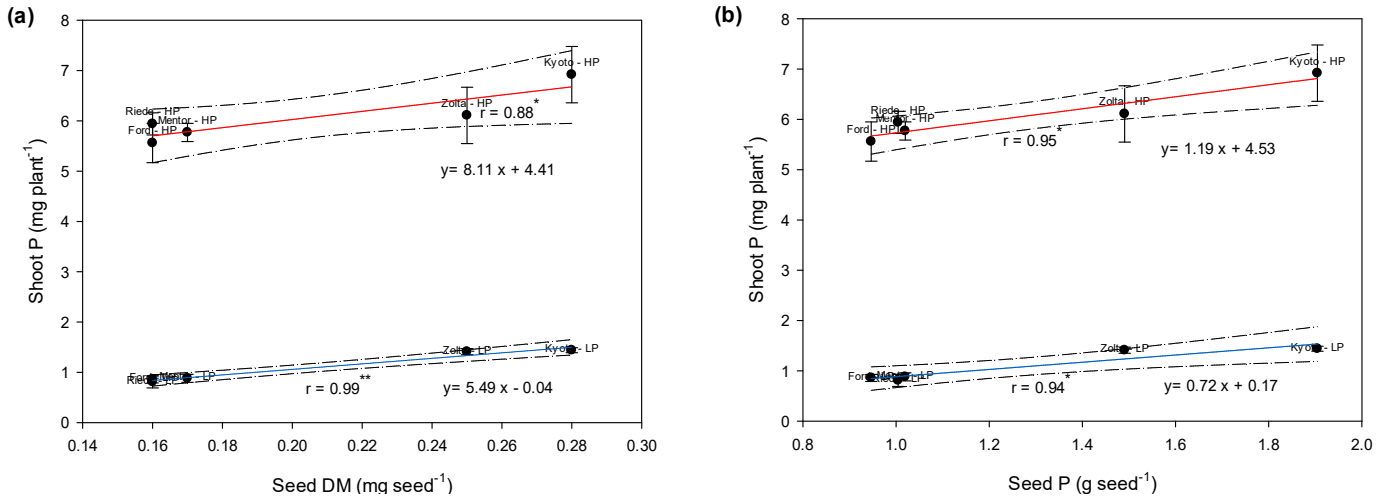


Fig. 19. Regressions of shoot P content on seed DM (a) and seed P content (b). Error bars represent standard errors.

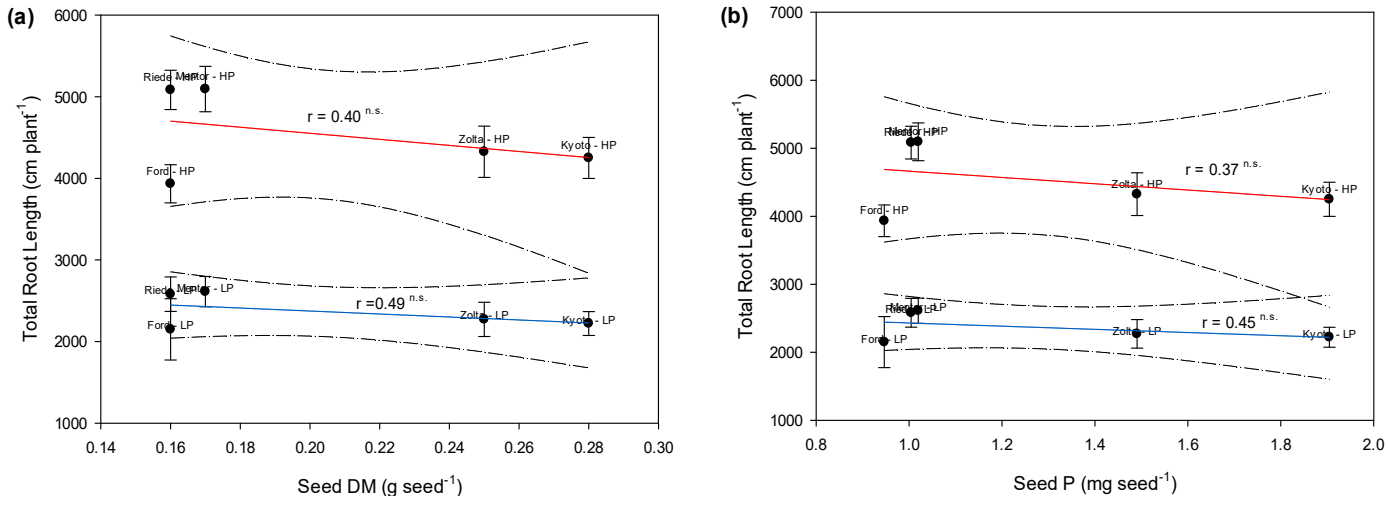


Fig. 20. Correlations of TRL on seed DM (a) and seed P content (b). Error bars represent standard errors.

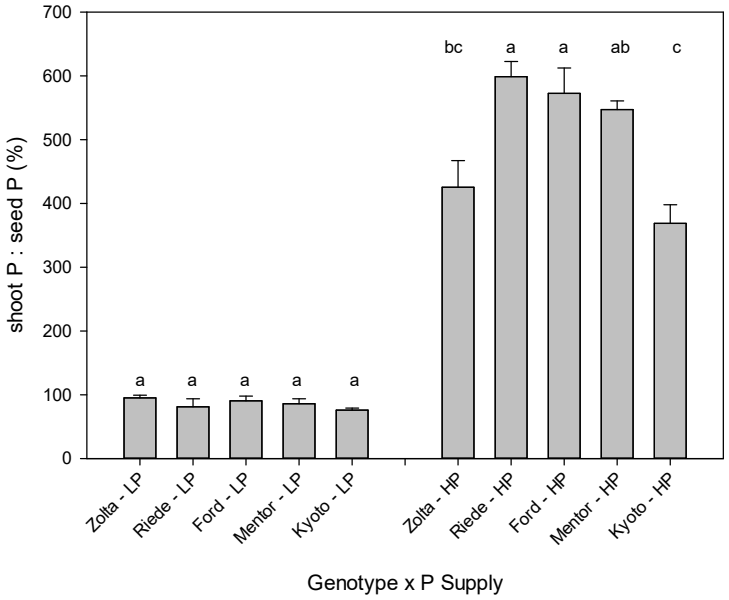


Fig. 21. Shoot P:seed P ratio (%) of the soybean genotypes at LP and HP, representing the relative contribution of seed P to shoot P uptake. The letters on top of each column represent Tukey's HSD grouping. Error bars represent standard errors.

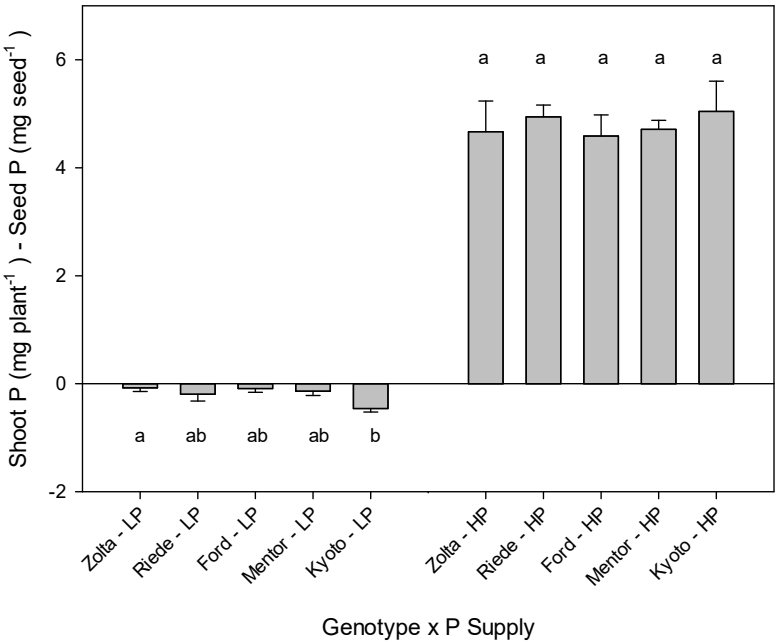


Fig. 22. Differences between shoot P uptake and seed P content of the soybean genotypes at LP and HP. The letters on top of each column represent Tukey's HSD grouping. Error bars represent standard errors.

As it is to see in Fig. 22, at LP all genotypes had less P in the shoot than in the seed - prior to sowing - with overall average of -0.19 mg at LP indicating that the seed P content was about the only source that provided P for shoot growth in all five soybean genotypes until 40 DAP. However, genotypes differed in the amount of apparent P transfer from seed to shoot. In Kyoto the difference between seed P and shoot P was significantly higher than in other genotypes at LP. At HP, in contrast, the differences between genotypes were not significant with an overall average of the difference between shoot and seed P at HP of 4.79 mg, indicating a substantial P uptake from the soil.

The variations in TRL at LP and HP are shown in Fig. 23. TRL was affected by both genotype and P supply effects, but the interaction was not significant. The increment of the substrate P from 7.20 mg $kg^{-1}$ at LP to 69.15 mg $kg^{-1}$ at HP increased TRL by 91%. This is close to the average increase in root DM from LP to HP by 84% (cf. Fig 23). A genotypic difference in TRL with a similar pattern at both LP and HP exists where Riede and Mentor attained more root length when compared to other genotypes. Nevertheless, variations in TRL between genotypes failed to explain the differences in shoot P content since the two variables do not correlate significantly (Fig. 27a). There is a trend for a negative correlation at LP, which might be a consequence of competition between shoot and root under P limited growth condition. However, TRL in general shows a positive effect on P uptake.

The average root diameter (ARD) for LP and HP was 0.39 mm and 0.41 mm, respectively, which is a marginal difference regarding the root morphological traits, but still reached the significance level ($p \leq 0.01$). The difference between the genotypes for ARD was also significant ($p \leq 0.05$) and the ARD showed a significant genotype x P level ($p \leq 0.01$) interaction. This interaction is mainly due to the behavior of Mentor, that showed the thickest roots at LP but the thinnest ones at HP (Fig. 24).

As shown in Fig. 25, we found differences in RSA between P supply levels ($p \leq 0.05$) and genotypes ($p \leq 0.001$) but no significant genotype x P level interaction. Based on the significance of genotypic effect on RSA, it is visible that Ford and Kyoto showed lower RSA values than the rest of the genotypes both at LP and HP. RSA also does not contribute significantly to explain the differences in shoot P content since the two variables do not correlate significantly (Fig. 27b). However, RSA in general shows a positive effect on P uptake.

In the present experiment the differences for SRL (the ratio of root length to root DM) between the genotypes were significant (Fig. 26), however this root character was not affected by the P supply. Therefore, the assumption of an increase in SRL at LP is rejected. At LP both Riede and Ford, showed more SRL than Zolta, whilst at HP Ford had more root length per root DM when compared to all other genotypes. The association between SRL and shoot P uptake

under both LP and HP was not significant (Fig. 27c). Accordingly, it does not seem that higher specific root length is more favorable for P acquisition either in low or high P environments. In contrast, there is a clear trend for a negative correlation at both P supply levels visible.

Soybean biomass and yield responses to P are well documented, but impacts on development are less defined. Fig. 28 shows the phenological development of soybean genotypes as affected by two levels of P supply. The LP treatment reduced the development rate of all genotypes. The differences of phenological stage (BBCH) between LP and HP became noticeable 20 DAP (about 380° Cd), and they increased as the cumulative thermal time proceeds. Amongst the genotypes Riede showed the strongest phenological response to the P application, i.e. the largest gap between LP and HP regarding the phenological stage. Furthermore, Zolta and Kyoto obtained the latest phenological stages at LP and HP, whilst Riede and Ford developed less far at both LP and HP.

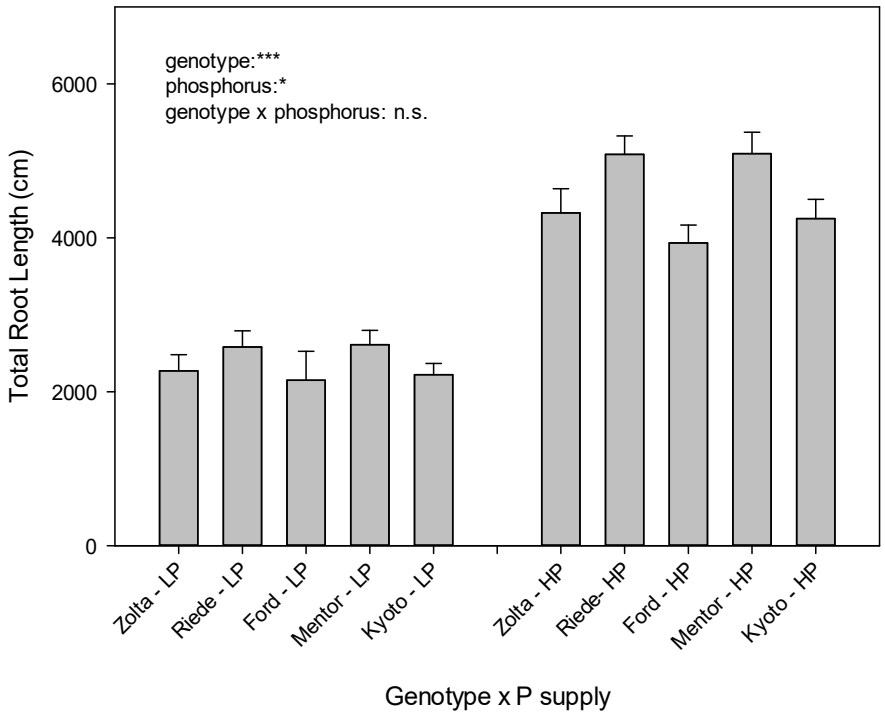


Fig. 23. Total root length (TRL) of the soybean genotypes at LP and HP. Error bars represent standard errors.

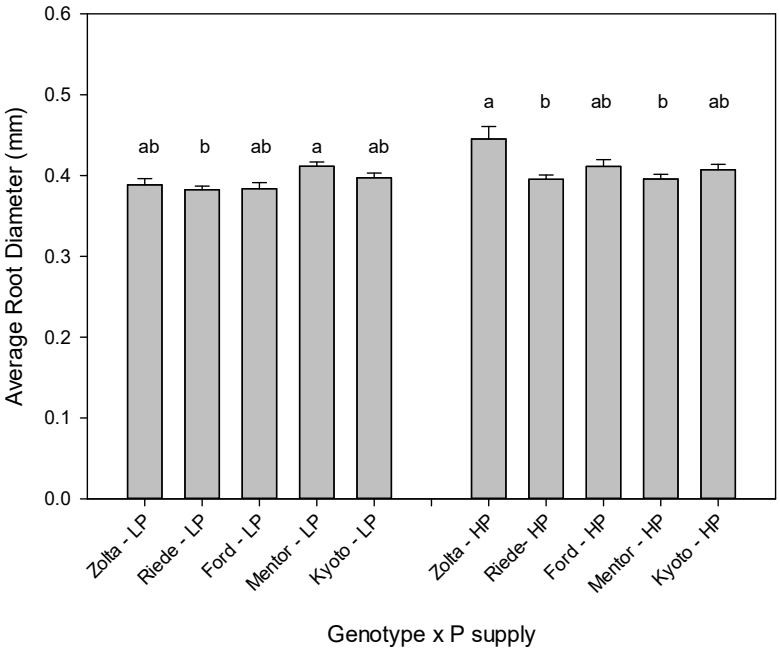


Fig. 24. Average Root Diameter (ARD) of the soybean genotypes at LP and HP. The letters on top of each column represent Tukey's HSD grouping. Error bars represent standard errors.

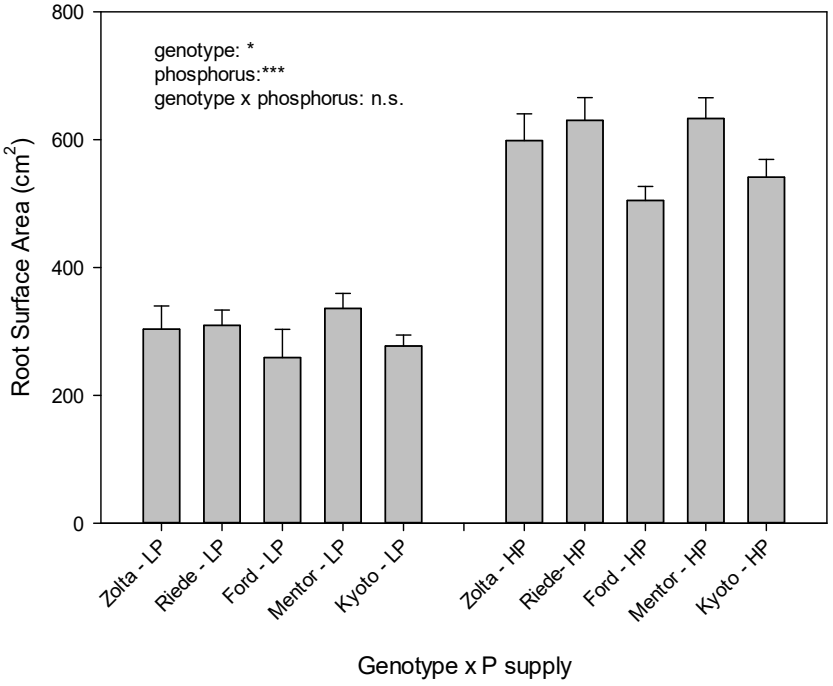


Fig. 25. Root surface area (RSA) of the soybean genotypes at LP and HP. Error bars represent standard errors.

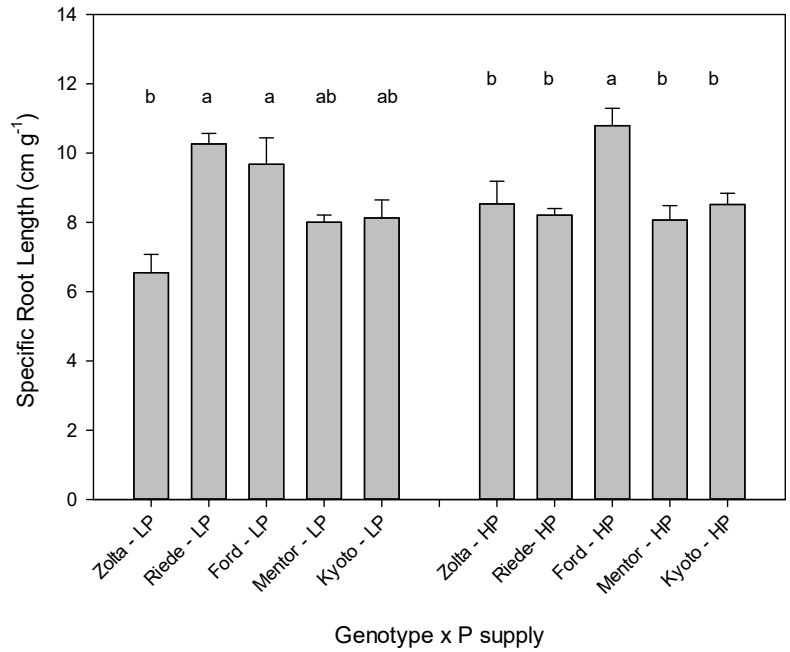


Fig. 26. Specific root length (SRL) of the soybean genotypes at LP and HP. The letters on top of each column represent Tukey's HSD grouping. Error bars represent standard errors.

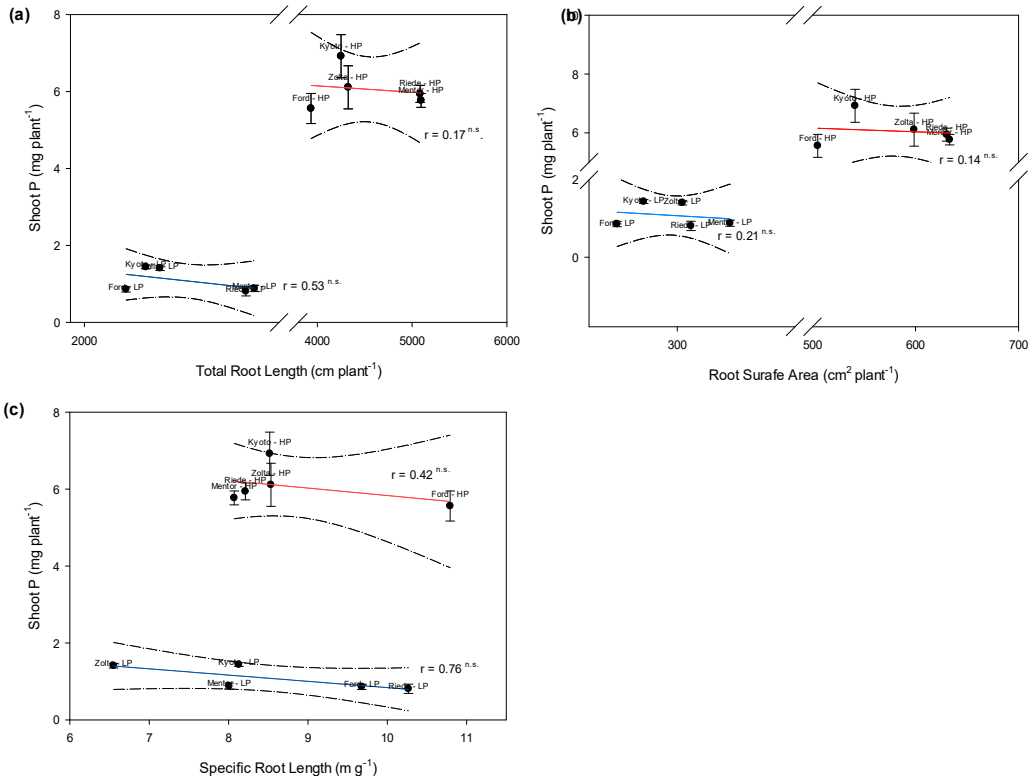


Fig. 27. Correlations of root morphological traits with shoot P uptake. Error bars represent standard errors.

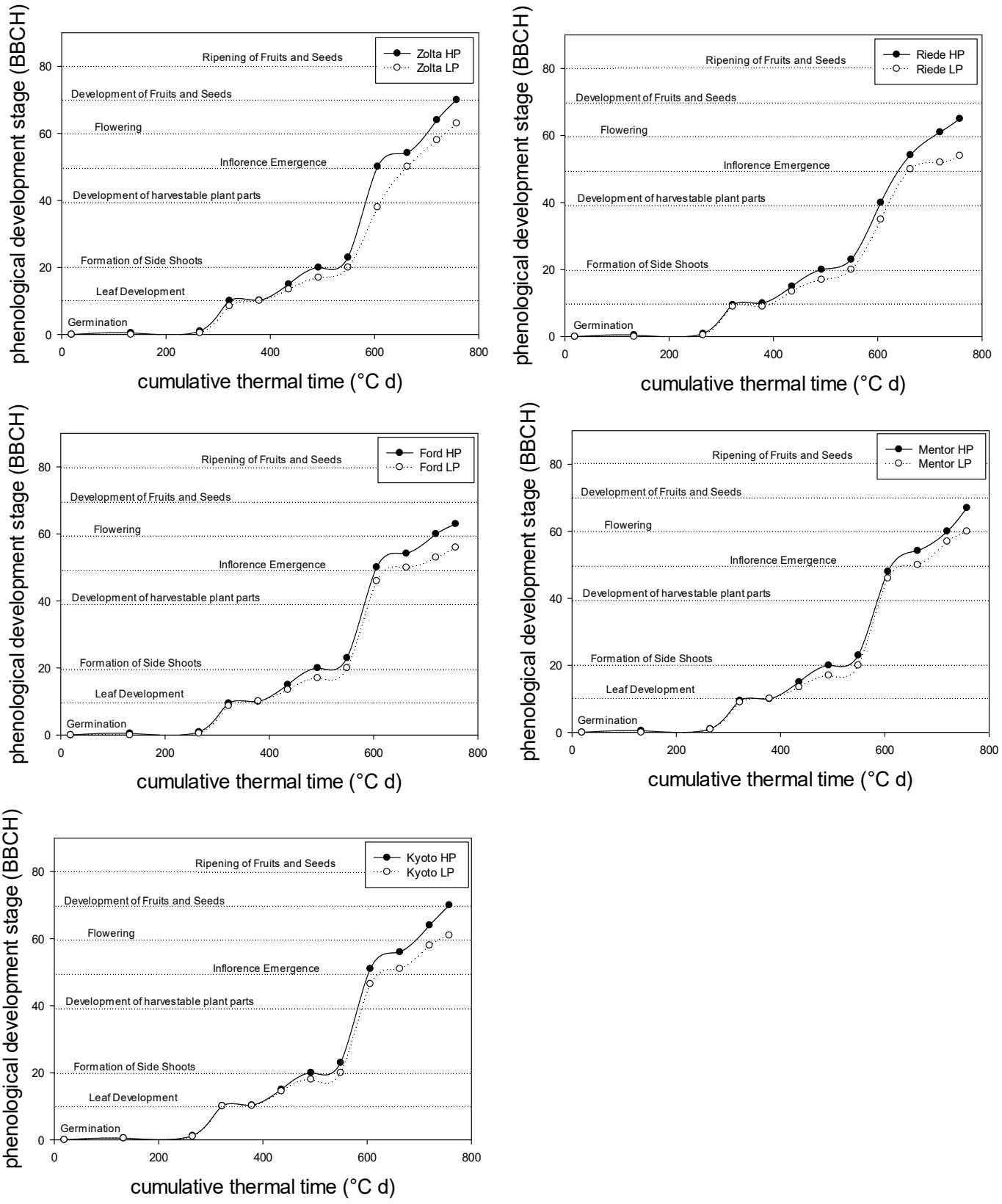


Fig. 28. Genotypic and P supply induced variation in phenological development of soybean cultivars. Cumulative thermal time (CTT) was calculated from planting day onwards.

## 4.3. Discussion of EXP1

We know from literature that a few days after planting the roots start to acquire P, nevertheless according to previous studies, it takes 4 weeks until the percentage of total plant P which has been uptaken by roots gets appreciable Veneklaas *et al*. (2012). Considering that the length of our experiment EXP1 was only 30 days, and the bioavailable P in the substrate was low, the present study aimed at the association between seed size and seed P reserves with shoot DM and P uptake.

Nadeem *et al*. (2012) have also proved that in maize, with even smaller seeds P content, the seed P alone can be enough for maximal seedling growth under low P phyto-availability for 4 weeks. Later on the genotypes with faster root establishment could more quickly complement seed-P content by root uptake.

In this experiment there has been a 1.7-fold variation in shoot DM, and a 2.8-fold variation in shoot P uptake among genotypes, which shows a substantial genotypic variation in aboveground biomass and P accumulation among the studied genotypes of soybean. These differences are positively, linearly correlated with seed weight and seed P content, implying that during early growth after germination, when the roots are not fully established, and under low P phyto-availability, the seed P reservoir is the primary source of nutrients and particularly of P for the shoot. Therefore, seed DM has to be considered when screening genotypes for fast early biomass accumulation.

Consistent with our findings that the shoot DM and also total DM of plants 30 DAP were genotypically different and closely (linearly) related to seed DM, Vandamme *et al*. (2016) also reported a moderate significant correlation under low P supply of seed size with shoot DM (r=0.75) and shoot P content (r=0.74) in soybean plants. For other leguminous crops like cow pea it has been also proven by Kang and Ofeimu (2012), that seed size had a significant effect on shoot and total plant DM. It is worth noticing that the overall shoot P concentration for all varieties was below 2 mg P $g^{-1}$ DM, which has been defined by White and Brown (2010) as critical tissue P concentration indicating that the plants presumably faced early stages of P-deficiency stress.

Seed size in the form of seed DM as well as seed P content differed significantly between the soybean genotypes. But also the seed P concentration was significantly variable in our set of soybean genotypes with a range of 5.76 mg $g^{-1}$ to 6.79 mg $g^{-1}$. Thomas *et al*. (2003) also showed that the concentration of phosphorus in soybean seeds generally varies from 5 mg $g^{-1}$ to 7 mg $g^{-1}$ DM. Considering that 7.5 mg $g^{-1}$ total P concentration in the seeds of soybean in an experiment by Veneklaas *et al*. (2012) has been reported for the seed fresh mass (FM), the range that we observed with our set of genotypes seems typical of the crop. Nevertheless, the

differences in seed P concentration explain only a small fraction of the variability in seed P content, while most of the differences in seed P content in this experiment were induced by the variations in seed DM. Mebrahtu *et al*. (1997) and Vandamme *et al*. (2016) have also reported that significant differences for seed P content among genotypes of soybean derived mostly from the variation in seed DM in opposite to seed P concentration. Additionally, the seed P effect on shoot biomass accumulation is confounded with effects of other nutrients which are also supplied by the seed reserves.

The positive yet weak correlations between the seed traits and root DM shows that nutrients are also invested in roots, but not as much as they are invested in the shoot which corroborates with previous results of Longer *et al.* (1986), who indicated that large seeds are superior to small seeds not only in shoot but also in root weight. Nevertheless, knowledge about the exact quantity of seed P allocation between the above and below ground organs demands analysis of the P concentration in the roots which was barely practical at the early growth stages of the plants considering the meager root biomass produced within 30 DAP.

We also showed that root DM varies under low P supply, but more root growth is hardly associated with higher shoot P. Therefore, it can be hypothesized that, with little P available in the substrate, more root growth for the young seedlings might increase the P acquisition but at the price of more metabolic costs for soil exploration which has accordingly flattened the slope of the regression line. Furthermore, the differences between genotypes in root traits such as total root length do not much contribute to explain the differences in shoot P content, as root P uptake plays no important role in P acquisition during the early phases of plant growth especially under low P phyto-availability.

Our research so far suggests that further work has to be carried out in order to evaluate the association of seed size and seed P reserves with shoot P uptake and crop growth under contrasting conditions of even more severe P stress vs. luxury supply of P and for a longer growth period beyond 30 DAP.

### 4.4. Discussion of EXP2

Among other traits, shoot and total plant biomass of soybean plants 40 DAP were highly correlated with seed size and seed P content at LP (Fig. 18), which supports the importance of seed P as a nutrient reserve for soybean establishment within the early growth stages. Thus they are important traits for low P tolerance in the early growth stages of soybean where the exogenous P is scarcely available. On the other hand, the variations in root biomass accumulation (Fig. 18 b and 18e) and in TRL (Fig. 20) were not dependent on the variations of seed weight and seed P content.

The soybean genotypes used in this study showed variability for all measured traits including biomass productivity and above ground P uptake, which could be successfully used to enhance the P status of the soybean plants on widely spread P-deficient soils. The effect of P supply on all traits except for SRL was significant as well, implying that the stress has been applied beyond the threshold and long enough to cause a significant decrease in yield and root morphological characteristics. According to Zang *et al*. (2011) also Mingshou *et al*. (2005), the increase of SRL is generally expected as an adaptation strategy to reduce the C costs for P uptake in plants that are grown under low P conditions. Bolan *et al*. (1987) also stated that the effect of P supply on specific root length is mediated through its effect on P status of the plant. Fernández *et al*. (2009) have also shown that the higher tolerance of soybean to P stress may be associated to a greater SRL. However, in contrast to that, Trindade & Araújo (2014) indicated that the soil P supply did not significantly affect specific root length. Furthermore the interaction of P supply and genotype seems to have great practical implications for biomass above and below ground as well as their components, also shoot:root ratio, TRL, SRL and shoot P:seed P(%). This variability for those traits for which the interaction was significant indicated different reactions among genotypes in response to phosphorus availability. E.g. in Riede it resulted in more biomass accumulation in the shoot and root at HP, or in Ford it increased the shoot:root ratio at HP.

In this experiment seed DM differed significantly between the soybean genotypes and four different seed size levels were obtained after the post-hoc comparison for 5 genotypes (Fig. 15a). The seed P concentration in Kyoto was significantly higher than in other genotypes, and all of the seed P concentrations (0.60%, 0.61%, 0.58%, 0.58% and 0.68% for Zolta, Riede, Ford, Mentor and Kyoto respectively) were within the range of seed P concentrations previously reported by Vandamme *et al*. (2016) in other soybean genotypes (0.37% to 0.74% P). Nevertheless, seed P concentrations explain only a small fraction of the variability in seed P content, while most of the differences in seed P content in this experiment were induced by the variations in seed DM. Mebrahtu *et al*. (1997) and Vandamme *et al.* (2016) have also reported that significant differences for seed P content among genotypes of soybean derived mostly from the variation in seed DM in opposite to seed P concentration. It is believed that soybean seed size is mainly controlled by genetic differences between varieties as shown by Krisnawati and Adie (2017), but according to Crawford and Williams (2018) it is also affected by environmental and cultural conditions during the seed filling period, which has been frequently referred in the literature as the parental effect. Parish and Bazzaz (1985) stated that seeds from a high nutrient parental environment are generally larger in size than seeds from a low nutrient parental environment.

Regarding early aboveground biomass accumulation at LP and HP, the genotypes can be classified into two groups. Genotypes with relatively higher shoot DM at LP (in the present study Zolta and Kyoto) might be considered as low P-tolerant while those with more shoot DM at HP (here: Kyoto and Riede, also to some extent Zolta and Mentor) will be the more P-efficient genotypes. At HP genotypes Riede and Kyoto accumulated more biomass in comparison to Ford (Fig. 16a).

Nevertheless, this higher level of early biomass accumulation in Riede was at the cost of P dilution in the shoot tissue (Fig. 17). The opposite of dilution, i.e. a comparatively high phosphorus accumulation, can be seen in Ford at HP, where less biomass accumulation at HP resulted in a significantly higher concentration of P, when compared to Riede (also Fig. 17). The tendency of “hyper-accumulation” of phosphorus in the shoot of the genotype Ford is in accordance with our findings in EXP1, where Ford accumulated more P in the shoot compared to other genotypes within a range of similar above ground biomass.

The genotypic differences of root DM at LP were not statistically significant, nevertheless Ford consistently had the least biomass in the underground organs at both LP and HP. Riede responded to the P supply with a root DM increase which was clearly above that of the other genotypes, as already observed with the aboveground biomass. Riede and also Mentor not only benefited from HP by high shoot biomass accumulation, but might also benefit from more P uptake in the later stages like seed filling by having more root biomass and a more extensive root system. The effects of roots on P nutrition requires further comprehensive investigations up to late growth stages of these soybean genotypes. Nevertheless, as suggested by Wang *et al*. (2004), it is the proportion of root hairs and fine roots (<0.05 mm diameter), which may play the most important role in P acquisition and not root biomass or total root length.

The differences in total DM of plants, i.e. the sum of the dry weights of root and shoot, were following the same trend as shoot DM because root DM was only a small fraction of total DM, therefore shoot DM is the leading factor regarding the total biomass accumulation 40 DAP under the given circumstances.

There are differences in shoot:root allometry among the five soybean genotypes which are consistent and similar to the results of our previous experiment, e.g. the lowest shoot:root ratio was observed in ES Mentor at both HP and LP treatments in the current experiment, which also showed the lowest ratio at an intermediate bioavailable substrate P concentration of 42 mg $kg^{-1}$ in the previous experiment. However, P supply had a strong positive effect on shoot:root allometry, which might indicate an improved root P uptake. This is in accordance with many similar studies; e.g. Ahlawat and Saraf (1982) stated that P supply enhances the shoot growth substantially but makes the root system also to some extent more extensive.

Nevertheless, its impact on shoot productivity is stronger, therefore it widens the shoot:root ratio. Vandamme *et al*. (2016) also stated that the root:shoot ratio (the reverse index) was generally higher at low P supply. But there are also other reports such as Gerardo *et al*. (2013), who found that severe P stress does not affect soybean shoot:root allometry compared to sunflower and maize.

A genotypic difference for TRL was found only between Riede and Ford (main effect) with a slightly stronger difference at HP. It is evident that variations in TRL are mostly impacted by root DM and there seems not much space for variation due to other morphological characteristics such as formation of cortical aerenchyma in roots or development of finer and longer roots per unit weight. The increasing differentiation of TRL with an increase in phyto-bioavailable P is similar with previous research results such as of Borkert and Barber (1985), who had shown that the total root length of soybean plants per pot is significantly higher in P-added treatments compared to the control without P. Maybe increased lateral root formation plays a role in increased TRL at HP. This is however in contrast with the findings of Gutiérrez-Boem and Thomas (1999), who observed an absolute increase in total root length under very low P supply (4.6 mg $kg^{-1}$ P) compared to a moderate P supply level of (32.3 mg $kg^{-1}$ P) in field grown soybeans. Jemo *et al*. (2017), also reported that P deprivation does not necessarily result in a significant root length decrease in legume crops such as cowpea.

The marginal difference of ARD at LP and HP that we observed in this experiment has been also reported by Fernández and Rubio (2015), who showed that soybean plants developed rather thinner roots at LP. According to Lynch (2015), this is a strategy of plants to decrease the metabolic costs for soil exploration. The genotypic variation of ARD in response to P supply indicated that ARD in some genotypes like Mentor decreased significantly in a way that the significant difference, which existed between Riede and Mentor at LP, faded at HP. For other genotypes, however, ARD either did not change much (Riede, Ford and Kyoto) or increased a bit as in the case of Zolta.

The variations in RSA were more explained by P supply than by genotype. The differences of genotypes showed a quite similar RSA pattern at LP and HP (interaction n.s.). On average, the genotypes at HP had 1.96 times more RSA compared to LP. According to McGrath *et al*. (2017), root surface area is a good indicator of nutrient uptake ability because it reflects the combined effects of TRL and ARD. Ojo and Ayuba (2013) also stated that RSA in soybean is a better measure of the absorptive capacity of a root system than TRL. Iman *et al*. (2006) had shown that increasing the soil fertility level from 50% to 100% of the conventional recommended rate increased the RSA in soybean by 79.2%, but an additional increment in fertilizers by 50% decreased the RSA. This suggests an optimum relationship of P supply and RSA. In contrast to the present results, Lambers *et al*. (2006) hypothesized that an increased

root surface area could be the result of compact cluster formation of short-branched roots or root hairs as a typical response to low bioavailability of P in the soil. In the present experiment, significant RSA differences between P supply levels (and to a smaller extent between genotypes) have been documented, but these do not contribute significantly to explain the differences in shoot P content (Fig. 27b). It seems that the differences in root morphological traits in such a set up do not necessarily play an important role in shoot P acquisition.

In spite of indifference of SRL to P stress, there were significant differences for SRL under both P supply levels between the genotypes with Ford having the highest root length per gram of root DM at HP and Zolta having the least SRL at LP. SRL has shown inconsistent responses to the nutrient supply in previous experiments. It has been claimed by Ho *et al*. (2005) that specific root length can change adaptively in reaction to the availability of nutrients. Julia *et al*., (2018) also Sunga and Gonzales (2015) found that the specific root length increased under P-limited conditions in rice or, as studied by Fernández and Rubio (2015) and Li *et al.* (2019), in maize. However, this is challenged by the findings of several other authors such as Løes and Gahoonia (2004), who did not observe any genotypic variation in specific root length with wheat and barley or Paponov *et al*. (1999), who found no change in specific root length in response to nutrient-poor conditions.

Correlation coefficients between soybean growth traits (shoot DM and total plant DM) and seed DM were similar to their correlation coefficients with seed P content, which supports findings of Vandamme *et al*. (2016) that shoot and total biomass were significantly and positively correlated with seed weight and seed P content. Nevertheless, we found no significant correlation for seed DM or rather seed P with root DM (Fig. 18b and 18e).

The significant linear regression between seed DM or rather seed P and shoot P uptake indicates that shoot P rises at both LP and HP as seed size, respectively seed P, increases. This is in accordance with findings of Vandamme *et al*. (2016), who reported a positive strong regression between seed size and shoot DM or shoot P content.

No correlation between seed weight and TRL has been found in this experiment (Fig. 20). Few researchers have addressed the correlation between seed weight or seed P with TRL. Our results differ to some extent from those of Vandamme *et al*. (2016), but in that experiment the roots were harvested only 13 DAP, when they are presumably more dependent on the seed nutrient reserves. Also in maize the correlation between seed weight and TRL has been already declined by Manavalan *et al*. (2012), indicating that TRL variations among genotypes were due to other inherent genetic variations and not affected by seed size.

The average shoot P:seed P ratio of 85.69% for the genotypes at LP, with no genotype exceeding 100%, indicates that soil P acquisition was negligible for all 5 genotypes at LP and

the P stored in the cotyledons of soybean was presumably the most important source for soybean plants until 40 DAP under severe P starvation. On the other hand, the average shoot P:seed P ratio of all genotypes at HP was 503%, ranging from 369% for Kyoto to 599% for Riede. Obviously, at HP there was a high contribution of soil P acquisition to shoot P. Interestingly Riede had the highest proportion of shoot P:seed P also in the previous experiment at a moderate P supply level, indicating that unless the P stress in very severe, Riede (followed by Ford and Mentor ) will be efficient in soil P acquisition.

At LP all genotypes of soybean in this experiment had less P in the shoot than they had stored in the seed, indicating that theoretically the seed P content alone is sufficient to provide the required P for survival of young soybean plants until anthesis under low P supply. This difference was larger (in absolute values) in the case of the big seeded variety Kyoto, which is in accordance with findings of White & Veneklaas (2012) also Veneklaas *et al*. (2012), who showed that up to 4 weeks after planting the total plant P is mostly provided by the seed P content. At HP, however the differences for shoot biomass accumulation of different genotypes are better projected, the effect of seed P on shoot biomass has diminished, since the external P supply to plants increased and the differences between genotypes regarding the role of seed P content became insignificant.

## 5. Conclusion

In the present study we have found variation in early biomass and P-accumulation in the aboveground organs of soybean genotypes during the vegetative development period grown at low P availability (LP). The differences between genotypes, however, were unexpectedly more pronounced at HP phyto-availability, and there were significant positive correlations between seed size or rather seed P content with shoot DM and Total DM at LP, as well as with P uptake at both LP and HP, underlining the importance of seed P content for improving P acquisition in soybean prior to anthesis specially at LP. There was additional significant genotypic variation in response to soil P supply at HP.

Genotypes Kyoto and Zolta, which had larger seeds and more seed P content, accumulated more biomass and relatively more P in their shoots at LP. Furthermore, among these two genotypes, the values for shoot DM, root DM and total DM as well as for shoot P uptake in Zolta at LP, were all located above the regression line for their relation to seed DM and seed P, while the same observations for Zolta at HP were below the prediction line. This indicates that Zolta used the seed P content better in the case of P deficiency than what is predicted on average for all genotypes included in this experiment. But for Kyoto all those values were

located exactly opposite to Zolta, suggesting that Kyoto was less productive in P uptake from the seed than predicted at LP, while at HP it was vice versa.

In response to phosphorus application, we found more genotypic diversity at HP and some genotypes do not follow the trend observed at LP. Namely Riede accumulated more biomass in the shoot at HP and to some extent also Mentor did so as well. This is also extendable to root DM and total DM, where Riede and Mentor benefited most from the P application.

Also the higher values for shoot P:seed P ratio at HP in Riede, Ford and Mentor imply that these genotypes were able to benefit better from the P applied to the substrate. But these variations could be hardly explained by differences in root traits as indicated by the weak correlations (cf. Fig. 27), nevertheless the two aforementioned genotypes Riede and Mentor developed more root DM and TRL at HP, while Ford has had the highest SRL among the genotypes at HP.

Shoot:root allometry also differed between genotypes and it decreased under P starvation as a strategy to enhance the P status in the aboveground organs. Mentor had the lowest shoot:root ratio both at LP and HP, but not all genotypes increased that ratio to the same degree at HP and Ford's value at HP was higher than those of the other genotypes.

Among root traits, we found differences between root DM, TRL, ARD, RSA and SRL between genotypes but they could not thoroughly explain the differences in shoot P content, approving that the differences in root morphological traits, which might result in different shoot P acquisition, are mostly covered up by the stronger effect of seed P in the first 4 weeks of plant growth.

Specifically, the observed variation in root DM, TRL, RSA and ARD under the contrasting P supply levels did not disclose genotypes with distinguished abilities in favor of overcoming P deficiency. Ford had comparably lower root DM, TRL and RSA at both HP and LP which could result in lower metabolic cost for soil exploration and a higher shoot:root ratio as mentioned before, but also less root expansion and suboptimal nutrient uptake from the soil.

The missing dependency of the shoot P uptake on TRL, RSA and SRL might be due to the comparatively short duration of the experiment. We know from Soleiman *et al*. (2007) that in other crops like wheat and canola with much smaller seeds and seed P content, significant positive correlations between the shoot P uptake and root length at 8 and 12 weeks after planting can occur. Also the chosen experimental set up with the use of fertigation in small pots might be a reason why plants with more root contact surface with the substrate do not necessarily show higher shoot P uptake, while such correlation could be expected in a field experiment with conventional fertilisation.

Moreover, a general significant increase in root length per unit of root mass (SRL) at LP, which could have been a strategy to cope with P starvation, was not confirmed. Nevertheless, with regard to root morphological plasticity and in contrast to the rest of genotypes like Ford, which had the highest SRL at both LP and HP, Riede showed relatively higher values of SRL at LP, indicating the formation of finer and longer roots per unit weight of root DM. A significant effect to enhance P acquisition in the case of soil P deficiency, however, could not be attested.

All in all, it can be concluded that seed weight largely affects shoot DM and total plant DM at LP supply, although no effect on root DM was confirmed. Also it can be stated that the shoot P content of plants 40 DAP is positively (linearly) related to seed weight and seed P content both at LP and HP, but soybean at HP supply took up much more P and produced much more dry matter than at LP. Additionally, differences in P acquisition between genotypes were more pronounced at HP, while no substantial genotypic differences were observed in P use efficiency under P deficiency. The larger differences between genotypes at HP could not be explained by any differences in root traits.

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