Phosphorus mobilizing capacity of selected grain legumes grown under phosphorus-deficient conditions

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ABSTRACT

Compared with other crops, legumes have superior ability to mobilize part of non-labile phosphorus (P) into labile/available forms. We explored the P-mobilization potential of selected grain legumes and its influence on growth, P uptake, and yield under P-deficient conditions. A pot experiment was carried out in a greenhouse with soybean (Glycine max L.), cowpea (Vigna unguiculata L.), green gram (Vigna radiata L.), and black gram (Vigna mungo L.) with two P fertilizer applications; without (P-deficient) and with (P-sufficient) Department of Agriculture recommended rates of triple super phosphate. Each crop was harvested at maturity and the bulk and rhizosphere soils were collected to quantify different P fractions using Hedley's sequential fractionation. Dry weight and P accumulation in different plant organs, i.e., roots, stems, leaves, and pods, were measured. Most plant organs maintained similar dry weight and tissue-P concentration irrespective of the P levels applied. All crops reduced their total plant-P uptake under P-deficient conditions. However, this did not make any change in either pod dry weight or P concentration of pods. Soybean and mung bean had a greater P accumulation compared to cowpea and black gram under both P levels. In contrast, cowpea and black gram showed improved P use efficiency (PUE) than other two legumes. Available P concentration (NaHCO₃-P) in rhizosphere soil is greater than that in bulk soil in soybean and cowpea whereas the rhizosphere NaHCO₃-P content in mung bean was greatly decreased compared to that of bulk soil when the crop was cultivated in P-deficient soil. The concentration of non-labile P fraction in rhizosphere of all grain legumes were significantly low compared to that of the bulk soil. Results suggest that selected grain legumes could be cultivated in P-deficient soil as they can adapt either through enhancing P uptake or increasing PUE.
INTRODUCTION

Phosphorus (P) is a key structural constituent of important molecules such as phospholipids, sugars, phosphates, and ribosomal RNA and plays a central role in energy transfer and metabolic regulation while serving as an essential element for protein synthesis (Veneklaas et al., 2012; Lambers and Plaxton, 2015). However, P is one of the least available macronutrients in many terrestrial and aquatic ecosystems (Hallama et al., 2019). The main reason for this is that P forms insoluble complexes with metal cations such as Al$^{3+}$, Ca$^{2+}$, and Fe$^{3+}$ present in the soil (Chen and Liao, 2016). Furthermore, organic-P in soil cannot be directly captured by roots, unless it is enzymatically hydrolyzed to liberate soluble P (Richardson et al., 2009; Dissanayaka et al., 2018). Application of P-fertilizers has been the most common management strategy to overcome crop-P deficiency that encounters in many cropping lands. This would not be a sustainable approach as global rock phosphate reserves, which are the major source of P fertilizers, are projected to exhaust by the end of this century. Moreover, up to 80% of applied P is fixed in the soil as insoluble forms while some amount runs off to aquatic and marine ecosystems (Lambers and Plaxton, 2015; Hallama et al., 2019). Agricultural P runoff has been identified as a primary factor in the eutrophication of lakes, rivers, and estuaries (Schoumans et al., 2014).

Plants employ a wide array of mechanisms to cope up with P-deficiency in their growing environment (Richardson et al., 2011; Simpson et al., 2011). The root foraging strategies, i.e., increased root length and root hair formation, enable plants to explore greater volumes of soil in search of P thereby acquiring more P from soil (Richardson et al., 2011). The P-mining strategies of plants facilitate desorption and mineralization of sparingly-available P and non-labile P pools in soil by means of P-mobilizing root exudates such as organic anions and phosphatases (Richardson et al., 2011; Simpson et al., 2011). The improved internal P-utilization efficiency of plants facilitates achieving higher yield per unit of P acquired in P-deficient soil (Richardson et al., 2011). Compared to other plant species, it has been well documented that the legumes are efficient in using “P-mining strategies” to increase P availability through exudation of P-mobilizing compounds (Richardson et al., 2011). Several legume species that secrete carboxylates and phosphatases to access sparingly-available and non-labile P have been examined. Some of those promising species are white lupin (Lupinus albus L.; Gardner and Boundy, 1983; Hocking and Randall, 2001; Cu et al., 2005), pigeon pea (Cajanus cajan L. Millsp.; Ae et al., 1990), faba bean (Vicia faba L.; Li et al., 2007), and chickpea (Cicer arietinum L.; Veneklaas et al., 2003). Furthermore, incorporation of legumes to farming systems is known as one of the efficient strategies for climate change adaptation as it naturally reduces the amount of inorganic nitrogen (N) fertilizers, reduces greenhouse gas emissions, and maintains soil fertility (Karkanis et al., 2018). The Department of Agriculture (DoA) recommends mung bean to cultivate as the third or fourth season crop in selected paddy tracts in the country. In addition, legumes are an integral component to increase the productivity of many cropping systems. Given these circumstances, the P-mobilization potential and P nutrition of Sri Lankan legume species particularly under P-deficient conditions has gained little or no attention. Therefore, the present study addressed this knowledge gap by examining P-mobilizing potential of four legume species, their growth and P nutrition both under P-deficient and -sufficient conditions.

METHODOLOGY

Experimental location, design, and treatments

The experiment was done in a naturally lit glass house at the University Experimental Station, Dodangolla belonging to the Mid Country Intermediate Zone (agro-ecological region: IM3a) in Sri Lanka. The experiment was done as a two factor factorial in Completely Randomized Design (CRD) with three replicates per treatment during December to March, 2020. Factor one was the P fertilizer application having two levels (application of P fertilizer as per DoA recommendation and without P fertilizer application). The factor two comprised four grain legume species comprising soybean (Glycine max (L) Merr; var. PB1), green gram (Vigna radiata L; var. M16), cowpea (V. unguiculata (L) Walp; var. Dhawala), and black gram (V. mungo L; var. MI1). In total, there were eight treatment combinations and 24 experimental units.

Preparation of growth medium, plant establishment, and management

Reddish Brown Latasolic soil was collected from top 15 cm layer in the University Experimental Station and mixed thoroughly with washed river sand in 1:2 w/w ratio to make the medium deficient in available P. The available-P and total-P of the medium were 11.8 and 129.4 mg P/kg, respectively. Total N, exchangeable potassium, and
pH of the medium were 0.9 mg-N/kg, 52.7 mg-N/kg, and 6.3, respectively. The soil was mixed with relevant rates of mineral fertilizers (basal dressing) according to the recommendations of the DoA. The soil moisture content was adjusted to 80% of the maximum water holding capacity. A bag with 30 cm height and 20 cm diameter was prepared with 300-gauge polythene and filled with 15 kg of prepared soil for each experimental unit. Seeds were obtained from the Field Crops Research and Development Institute, Mahaluppallama, Sri Lanka. Four uniform seeds were established in each bag. After one week of germination, seedlings were thinned to have two uniform plants per pot. Watering was done regularly to maintain soil moisture at 80% of the field capacity.

**Harvesting of plants and rhizosphere soil collection**

Plants were harvested at physiological maturity. During harvesting, shoot was cut from the base of the stem and was partitioned to stems, leaves, and pods. Root system was lifted carefully out from the soil and then shaken gently to remove loosely adhering soil. The rhizosphere soil was collected by vigorous shaking of the root system, followed by gentle brushing without damaging the root system. Rhizosphere soil was sieved through 2 mm holes to remove the remaining root parts. After rhizosphere soil collection, roots were washed thoroughly to remove the adhering soil. Shoot and root samples were then oven-dried at 70 °C until constant weight and dry weights (DW) of each plant part were measured. All plant samples were ground and digested in H₂SO₄-H₂O₂, and the P concentration in the digested solution was quantified using vanadomolybdate blue method (Murphy and Reley, 1962).

**Analysis of different P fractions in rhizosphere and non-rhizosphere soil**

For the determination of rhizosphere-P fractions, Hedley’s sequential fractionation (Hedley *et al.*, 1982) was used with some modifications. Three P pools, namely, available P (NaHCO₃-P), sparingly-available P (NaOH-P), and unavailable P fractions in the rhizosphere and bulk soil were measured. Quantities amounting to 0.5 g of rhizosphere and bulk soil were extracted sequentially by shaking overnight (16 h) firstly with 30 ml of 0.5 M NaHCO₃ after adjustment to pH 8.5 and secondly, with 30 ml of 0.1 M NaOH. Total P was quantified separately using vanadomolybdate blue method (Murphy and Reley, 1962) after digestion of the samples with H₂SO₄-H₂O₂. The concentration of non-labile P fraction was calculated as the difference between total P and the sum of available and sparingly-available P.

**Data analysis**

Data analyses were performed with SAS computer software (SAS ver. 9.1; SAS Institute Inc., Cary, NC, USA). Biomass and P concentration of different plant organs, P fractions in rhizosphere and non-rhizosphere soils were subjected to two-way ANOVA using the P supply and legume species as the treatment effects. Significance of the difference between bulk soil and rhizosphere P fractions were assessed using Tukey’s Studentized Range Test at P=0.05. Treatment means are presented in this paper with the standard error.

**RESULTS AND DISCUSSION**

**Growth response of selected legumes under two P application rates**

Dry weights of plant organs were significantly different for the crop type whereas P level and interaction effects were non-significant (P>0.05; Table 1). Soybean and black gram produced more root dry mass compared to those of cowpea and mung bean. For example, across two P levels, root DW of soybean was higher by 84% and 88% than cowpea and mung bean, respectively. Similarly, black gram produced 80% and 85% greater root DW compared to those two legumes (Table 1). Production of greater root biomass could increase nutrient uptake from the soil. As P bioavailability is generally greatest in the topsoil layer, topsoil foraging is known to be an efficient strategy to increase P uptake, which could attain through increased production of axial roots, greater lateral root density, and greater number of root hair and its length (Lynch and Brown, 2001; Lynch, 2019). For example, common bean (*Phaseolus vulgaris* L.) cultivars with greater biomass of basal roots were capable of acquiring more P and producing greater yield under P-deficient stress than cultivars with fewer number of basal roots (Walk *et al.*, 2006; Rangarajan *et al.*, 2018). In the present study, each cultivar produced similar root biomass both under P-deficient and sufficient condition (Table 1) indicating that the root growth was not limited by P-deficiency.

Across two P levels, the highest stem DW was produced by black gram whereas the lowest was recorded in mung bean (Table 1). Highest leaf DW was observed in black gram grown with P supply at DOA recommendation whereas the lowest was from cowpea grown without external P supply. Furthermore, leaf DW of mung bean and black gram was higher compared to soybean and cowpea. Across the two P levels, pod DW was in the descending order of mung bean > black gram>
soybean> cowpea. Interestingly, P levels had no significant influence (P>0.05) on plant growth and yield formation process. Therefore, it is reasonable to suggest that the all legumes used in present study could adapt to P-deficient condition, which could have probably been achieved partly through similar root growth across P-deficient and -sufficient conditions, helping the plants to explore similar volumes of soil for P.

Table 1. Dry weight of different plant organs of selected grain legumes grown under two P levels. Values represent the means of three replicates ± SE (standard error)

| Legume species | P level   | Dry Weight (g/plant) |                |                |                |
|----------------|-----------|----------------------|----------------|----------------|----------------|
|                |           | Root     | Stem    | Leaf   | Pod   |
| Soybean        | Deficient | 2.43±0.16 | 7.45±0.31 | 4.65±1.08 | 14.02±0.23 |
|                | Sufficient| 2.81±0.11 | 7.07±0.26 | 4.47±1.42 | 15.78±0.48 |
| Cowpea         | Deficient | 1.39±0.04 | 6.51±0.21 | 4.32±0.33 | 9.48±2.09  |
|                | Sufficient| 1.46±0.08 | 7.04±0.70 | 4.93±0.37 | 11.72±0.83 |
| Mung bean      | Deficient | 1.60±0.17 | 6.55±0.77 | 6.95±0.40 | 15.56±2.28 |
|                | Sufficient| 1.18±0.23 | 6.48±0.55 | 6.36±0.60 | 20.16±1.66 |
| Black gram     | Deficient | 2.49±0.23 | 9.31±0.46 | 5.86±1.44 | 16.34±1.40 |
|                | Sufficient| 2.65±0.19 | 9.81±1.12 | 7.65±0.25 | 16.29±1.34 |

P values in the table are related with the two-way analysis of variance for the factors, variety, P level and interaction of variety x P level had no significant impact on the parameters (P>0.05)

Table 2. P concentrations of different plant organs of selected grain legumes grown under two P levels. Values represent the mean of three replicates ± SE (standard error)

| Legume species | P level   | Tissue P concentration (mg P/g DW) |                |                |                |
|----------------|-----------|-----------------------------------|----------------|----------------|----------------|
|                |           | Root     | Stem    | Leaf   | Pod   |
| Soybean        | Deficient | 0.60±0.08 | 0.43±0.06 | 0.61±0.02 | 3.04±0.12 |
|                | Sufficient| 0.44±0.14 | 0.39±0.08 | 0.80±0.11 | 3.27±0.22 |
| Cowpea         | Deficient | 1.07±0.17 | 0.73±0.23 | 1.08±0.09 | 1.92±0.03 |
|                | Sufficient| 0.97±0.02 | 0.59±0.11 | 1.19±0.34 | 2.13±0.12 |
| Mung bean      | Deficient | 1.07±0.13 | 0.78±0.16 | 0.93±0.18 | 2.20±0.14 |
|                | Sufficient| 0.90±0.27 | 0.97±0.15 | 1.08±0.10 | 2.28±0.08 |
| Black gram     | Deficient | 0.80±0.10 | 0.66±0.02 | 0.67±0.04 | 1.87±0.03 |
|                | Sufficient| 0.58±0.15 | 0.82±0.09 | 0.82±0.05 | 1.78±0.03 |

P values in the table are related with the two-way analysis of variance for the factors; legume type, P level and interaction of legume type x P level had no significant impact on the parameters (P>0.05)
Distribution of P among different plant organs and use efficiency of acquired P

Similar to dry matter production, P concentration of different plant organs did not change with P level (P>0.05; Table 2). Accumulation of P in various plant parts depended solely on the legume type. Among all plant organs, P concentration of pods was higher than those of roots, stem and leaves for all legumes. The highest P concentration of root was found in cowpea while soybean produced the lowest. The P concentration of stem was comparatively lower in soybean compared to cowpea, mung bean and black gram. Cowpea had the highest leaf-P concentration among all legumes. Moreover, the greatest P loading to pods/grains was observed in soybean whereas black gram accumulated comparatively lower P in its pods. Although plant-P concentration in many crops is reported to be greater when P is supplied to growth medium compared to the plants cultivated on P-deficient medium (Maltais-Landry, 2015), the results of this experiment contradicted from this phenomenon as P accumulation in different plant parts was similar among P-deficient and -sufficient conditions. Probable reason for this discrepancy could be the difference of soil-available P concentration in P-deficient treatment. Maltais-Landry (2015) used resin P of 5 mg P kg⁻¹ as P-deficient treatment whereas in present study, the available P concentration of 11.8 mg P kg⁻¹ was used to represent P-deficiency condition. Generally, NaHCO₃-P concentration is greater than resin-P in soil as resin-P contains only the P which can be extracted by water (Hedley et al. 1982). Therefore, there might not be a significant difference of resin-P concentration of P-deficient treatment of the present study compared to that reported by Maltais-Landry (2015). Hence, this should be the ability of the different legume species used in the present study to withstand P-deficiency conditions by means of employing adaptation strategies.

To survive under P-deficient conditions, plants need to either increase P acquisition or improve the use efficiency of acquired P (Lambers et al., 2010; Richardson et al., 2011; Veneklaas et al., 2012; Stigter and Plaxton, 2015). In the present study, the effects of P level and legume species were significant (P<0.05) on total P uptake of crops (Table 3). Soybean and mung bean accumulated more plant P compared to cowpea and black gram under both P levels. The total P uptake of all legumes except black gram showed a significant increase under P-sufficient condition compared to P-deficient condition (P<0.05; Table 3). For example, total plant P uptake with P supply to soybean, cowpea, mung bean were 17%, 24%, and 25% greater, respectively, compared to those under P-deficient soil.

The P use efficiency (PUE) is defined as the amount of biomass produced per unit of P accumulated. The legume type had a greater influence in determining the PUE whereas P treatment did not remarkably change PUE of any legume (Table 3). The lowest P uptake was recorded by cowpea and the highest PUE was attained by black gram. The PUE of black gram across two P levels showed a significant increase (P<0.05) than those of other three legumes used in the study. These results suggest that the black gram have employed efficient means to use acquired P in biomass formation. Kugblenu et al. (2014) demonstrated that some cowpea varieties, which are highly tolerant to P-deficiency stress, had lower yield reduction compared to the plants grown under sufficient P supply.

Even in this study, none of the grain legumes showed a significant reduction in their yield despite the reduction in total plant P uptake under P-deficient condition. The results also highlighted that selected legumes have the ability to efficiently utilize acquired P in biomass formation and load sufficient amounts of P to grains even under P-deficient conditions. Plants use different means to increase the use efficiency of acquired P when they are exposed to P-deficiency in their growing environment. For example, remobilization of P from different cellular components of mature/senescing organs to young growing tissues helps plant to grow well when P uptake cannot fulfill the demand (Dissanayaka et al., 2018). Replacement of membrane phospholipids by lipids that do not contain P, could save P for other cellular processes when plants are grown under P-deficiency. Moreover, vacuolar purple acid phosphatases scavenge P from intracellular P-containing compounds during prolong P-deficiency (Feder et al., 2020; Luo et al., 2020). Some of these mechanisms might have come into effect in determining the enhanced use efficiency of acquired P by black gram.

Dynamics of different P fractions in rhizosphere

In the present study, rhizosphere soil-P fractions were sequentially separated into three pools namely, available or labile, sparingly-available, and non-labile. Phosphorus that can be extracted with NaHCO₃ is regarded as labile soil-P fractions and known to be the most available form for plant uptake (Bowman and Cole, 1978; Tiessen and Moir, 1993). The NaOH-extractable P is regarded as sparingly-available P pools (Hedley et al., 1982;
and the bulk soil in the other legumes tested, except in cowpea grown under P-sufficient conditions (Fig. 2). In contrast, the concentration of non-labile P pools significantly decreased in the rhizosphere of all legumes (Fig. 3). For example, reduction of concentration of non-labile pool in rhizosphere compared to bulk soil in soybean, cowpea, mung bean, and black gram under P-deficient condition was respectively, 42%, 34%, 41%, and 24%. Relevant reduction under P supplied conditions was approximately around 32% for all legumes used in the present study (Fig. 3).

Table 3. Total P uptake and P use efficiency of selected grain legumes grown under two P levels. Values represent the means of three replicates ± SE (standard error)

| Legume species | P level   | Total plant P uptake (mg/plant) | P use efficiency (g-DW/mg-P) |
|----------------|-----------|--------------------------------|-----------------------------|
| Soybean        | Deficient | 50.29±1.78                      | 0.57±0.03                   |
|                | Sufficient| 58.95±4.37                      | 0.52±0.05                   |
| Cowpea         | Deficient | 28.98±3.87                      | 0.75±0.03                   |
|                | Sufficient| 36.17±3.33                      | 0.70±0.05                   |
| Mung bean      | Deficient | 47.95±7.53                      | 0.66±0.05                   |
|                | Sufficient| 60.06±4.03                      | 0.57±0.03                   |
| Black gram     | Deficient | 42.58±1.10                      | 0.80±0.03                   |
|                | Sufficient| 45.03±4.74                      | 0.81±0.03                   |

When P is deficient in growing environment, roots employ both morphological and physiological mechanisms to enhance uptake of limited P. While increasing the root mass including lateral roots, root hairs help exploring greater volumes of soil in search of P, root exudation of P-mobilizing compounds such as malate and citrate is an effective physiological strategy to enhance the concentration of available P fraction, which is attained through mobilizing P either from sparingly-available forms or non-labile forms (Richardson et al., 2011; Simpson et al., 2011; Lambers et al., 2013; Lynch, 2019). The latter mechanism could have been the most relevant reason behind the changes observed in three different P pools between rhizosphere and bulk soil in the present study (Figs. 1, 2, and 3). Moreover, the increased concentration of available P pool in rhizosphere soil might have been the reason to the enhanced mobilization of P from non-labile P fraction. Many legumes are well adapted to P-impoverished environments and have demonstrated this promising strategy to survive under those environments. Previous findings also showcased that some legumes are capable of mobilizing P from non-labile pool than their own requirement, thus enabling the neighboring/co-cultivated species also to have access to part of the mobilized P by legumes (Li et al., 2003, 2004, 2007; Cu et al., 2005; Dissanayaka et al., 2015). The P-mobilizing potential of grain legumes identified in this study could be used to increase P availability in soils where a considerable part of P is remained in non-labile form. Among the legumes selected for the study, mung bean is recommended to be cultivated as the third season crop in selected paddy tracts in the dry zone of Sri Lanka purely...
Figure 1. Concentration of available P fraction (NaHCO\textsubscript{3}-P) in bulk and rhizosphere soil of selected grain legumes grown under two P levels. Different letters within each legume denote significant differences between treatments (P≤0.05). Vertical lines represent the standard error (S.E.; n=3). (P-, P-deficient conditions, P+, P-sufficient conditions)

Figure 2. Concentration of sparingly-available P fraction (NaOH-P) in bulk and rhizosphere soil of selected grain legumes grown under two P levels. Different letters within each legume denote significant differences between treatments (P≤0.05). Vertical lines represent the standard error (S.E.; n=3). (P-, P-deficient conditions, P+, P-sufficient conditions)
Figure 3. Concentration of unavailable P fraction in bulk and rhizosphere soil of selected grain legumes grown under two P levels. Different letters within each legume denote significant differences between treatments (P≤0.05). Vertical lines represent the standard error (S.E.; n=3). (P-, P-deficient conditions, P+, P-sufficient conditions)

aiming to utilize the residual moisture after rice cultivation (Amarasingha et al., 2017). Hence, future exploration into P nutrition of paddy crop cultivated after mung bean would be important to determine the nutritional benefit of this cultivation practice to the rice-legume rotational cropping system.

CONCLUSION

The results of the study suggest that two soil-P levels had no significant influence on dry matter accumulation of roots, stems, leaves and pods of the four grain legume crops. Furthermore, all plant organs maintained a similar tissue P concentration irrespective of the P level. However, the response of legumes to the P levels in soil varied with the species. All four legumes used in the study significantly reduced unavailable P fraction in their rhizosphere. The P-mobilizing potential of grain legumes identified in this study could be used to increase P availability in soils where a considerable part of P is remained in non-labile forms.

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