Rhizosphere processes do not explain variation in P acquisition from sparingly soluble forms among *Lupinus albus* accessions

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*Key words: carboxylates, cluster roots, pH, phosphate, rhizosphere*

*Running title: Lupinus albus variation in sparingly soluble P use*
Summary

Seven *Lupinus albus* L. landraces were selected, based on their geographic origin and the soil type and pH at the site of collection of the seeds, and compared with the cultivar Kiev mutant. We hypothesised that those landraces collected from red/yellow, acidic sands (pH 5-5.7) would be better at accessing P from FePO$_4$ or AlPO$_4$ than those selected from brown, neutral (pH 7) or fine, calcareous, alkaline sands (pH 9), and that those selected from fine, calcareous sands would be more effective at acquiring P from Ca$_5$OH(PO$_4$)$_3$. Plants were grown in sand and supplied with 40 mg P kg$^{-1}$ as the above sparingly soluble forms, or as soluble KH$_2$PO$_4$; control plants received no P. All genotypes were able to access these P sources. Variation in accessing poorly soluble P was not due to differences in rhizosphere carboxylate concentration, cluster-root development or rhizosphere-extract pH. *L. albus* landraces with a better ability to use P from different sparingly soluble forms could be exploited to develop cultivars that are more P-acquisition efficient on soils that are low in [P] or highly P-sorbing; however, desirable genotypes cannot simply be selected for based on soil type of origin.
**Introduction**

Soils characterised by poor phosphorus (P) availability for agricultural purposes are widespread globally (see review by Raghothama and Karthikeyan 2005). For these soils to be agriculturally productive, they require application of large quantities of P as manufactured, water-soluble superphosphate or ammonium phosphate fertilisers. Most soluble phosphate applied to P-deficient soil is retained by iron, aluminium or calcium ions exposed at surfaces of soil particles, and dissolution of sorbed P decreases with increasing time since P application (Barrow 1980). Sparingly soluble P forms are virtually unavailable to *Triticum aestivum* L. (wheat), relative to its availability to some legume species, including *Lupinus albus* L. (Bolland et al. 1999; Nuruzzaman et al. 2005). Therefore, the use of P-acquisition efficient species such as *L. albus* is a strategy to improve the P efficiency of farming systems on soils where P is poorly available.

The release of carboxylates from roots increases the concentration of inorganic P (P_i) in the rhizosphere, because carboxylates compete with P_i for binding sites on soil (Gerke 1992). The formation of root clusters, which exhibit an exudative burst of carboxylates, is an adaptation that allows *L. albus* to access sparingly soluble forms of P (reviewed by Shane and Lambers 2005). There have been many studies on physiological aspects related to P uptake by *L. albus*; however, these are typically studies dealing with only one genotype (e.g., Gardner et al. 1982a; Gerke et al. 1994; Shane et al. 2003).

Some studies have explored tolerance to calcareous soils among Egyptian genotypes, which display a greater capacity to grow in calcareous soil than European genotypes (Raza et al. 2001; Kerley et al. 2002; Kerley and Huyghe 2002). Liu and Tang (1999) found variability among *L. albus* genotypes in capacity to grow in limed or alkaline soils.
Variation in ability to access sparingly soluble P forms occurs among species of the genus *Lupinus* (Pearse *et al*. 2007). Currently very little is known about genotypic variation in P-acquisition efficiency within the species *L. albus*. The study of release of carboxylates and the relative ability of different landraces within a species to access sparingly soluble P$_i$ may identify variation that could be exploited to develop cultivars that are more P-acquisition efficient on soils that are highly P-sorbing. Seven *L. albus* landraces were selected, based on their geographic origin, and on the soil type and soil pH of the site where the accessions were collected; we included the cultivar Kiev mutant, as this genotype has been used in many studies. We hypothesised that those landraces selected from red/yellow, acidic sands (pH 5, 5.4 or 5.7) would be better at using P from FePO$_4$ or AlPO$_4$ than those selected from brown, neutral (pH 7) or fine, calcareous, alkaline sands (pH 9); conversely, those selected from fine, calcareous sands would be more effective at using P from Ca$_5$OH(PO$_4$)$_3$. 
Materials and Methods

Selection of accessions

The *L. albus* L. accessions were selected based on differences in soil type of origin and geographic location as shown in Table 1. The accessions included the cultivar Kiev mutant and several landraces (collection held by the Department of Agriculture and Food, Western Australia).

Plant culture

Plants were grown in 3 kg of sterilised, washed river sand (*n* = 4). The river sand (particle size range 0.2-2 mm) was analysed for properties by CSBP FutureFarm analytical laboratories (Bibra Lake, WA, Australia). Properties of the river sand are listed in Table 2, together with the procedures used to measure these properties. The sand was weighed and placed into tumble jars with either 0 or 40 mg kg$^{-1}$ of P in the form of either AlPO$_4$, FePO$_4$, Ca$_5$OH(PO$_4$)$_3$ or KH$_2$PO$_4$. The jars were placed in a soil tumbler, and tumbled 400 times to mix in the P. The sand was then transferred to sealed black plastic pots lined with polyethylene bags. A single jar was used for each treatment. All other added nutrients were, in mg per kg of sand, as follows K, 103.3; S, 49.1; Ca, 57.2; Mg, 1; Cu, 0.5; Zn, 2; Mn, 4; B, 0.12; Co, 0.084; Na, 0.21; Mo, 0.44; Fe, 5.5; Cl, 3; and N, 40; the nutrients were dissolved in water and then applied to each pot, wetting it up to 70% of field capacity. N was resupplied after sowing at a rate of 40 mg kg$^{-1}$ every 2 weeks in the form of Ca(NO$_3$)$_2$ to prevent N limitation and nodulation, and thus ensure that differences in biomass were due to differences in P uptake only.
Eight *L. albus* accessions were sown at two plants per pot on 17 February 2005, and thinned to one plant per pot 6 days after sowing. Unplanted pots were also included as a control. The plants were watered to weight (corresponding to 70% of field capacity) every 2 days for 4 weeks, and then watered to weight daily for the final 3.5 weeks. The plants were grown in a temperature-controlled glasshouse (20°C day/15°C night) and received approximately 60% of outdoor late summer light at latitude 31°60’S. The pots were randomly relocated weekly. The plants were harvested in a single harvest when visual differences in growth between P treatments were observed, before flowering had occurred.

*Plant measurements*

Genotypes were harvested by replication to ensure ceteris paribus conditions applied to exudate collection. Intact plants were removed from the plastic pots, and plastic bags surrounding the roots in sand were cut away to provide minimum disturbance to the roots. The plants were then gently lifted from the sand, and lightly shaken to remove bulk sand from the root systems. The roots were transferred into either 100 or 250-ml vials, depending on their size, and a measured amount of 0.2 mM CaCl$_2$ was applied, ranging from 30 to 150 ml dependent on root mass such that the whole root system would be covered when immersed. The roots were then gently dunked to remove as much rhizosphere sand as possible for 15-90 seconds depending on size. Care was taken to minimise root damage; however, it cannot be excluded that a small amount of carboxylates may have originated from cellular damage. After the roots were removed, the containers with the rhizosphere sand and root-washing solution were shaken by hand,
and the pH of the extract was measured (Ionode IJ44 pH electrode, Tennyson, Queensland, Australia). A subsample of the extract was then filtered through a 0.2-µm syringe filter into a 1-ml HPLC vial. The extract was acidified with a drop of concentrated phosphoric acid, and placed on ice until being transferred to a -20 °C freezer until HPLC analysis. The root systems were then washed thoroughly to remove any remaining sand.

Fresh and dry weights of roots (cluster roots were separated from the rest of the roots), stems and leaves (senescent leaves harvested separately) were recorded. All cluster roots, including juvenile, mature and senescent ones, were sampled. A cluster root comprises a primary lateral root with defined clusters of more than 10 secondary lateral roots per centimeter (Johnson et al. 1996). The leaves, roots and stems were dried in an oven at 70°C for 10 days, and placed in a desiccator until dry weights were recorded. To determine the P concentration in the tissue, three mature leaves of similar age and size were selected. Approximately 200 mg was digested using a nitric/perchloric acid mixture, and the digest was analysed using the molybdo-vanado-phosphate method (Kitson and Mellon 1944).

Carboxylate analysis

Analysis of 100 µl injections of rhizosphere extracts was performed by HPLC (Cawthray 2003) using a 600E pump, 717plus autosampler and a 996 photodiode array detector (Waters, Milford MA, USA), and an Alltima C-18 reverse-phase column (250 mm * 4.6 mm I.D.) with 5-µm particle size (Alltech Associates, Deerfield, IL. USA). The working
standards included malic, malonic, lactic, acetic, maleic, citric, *cis*-aconitic, succinic, fumaric and *trans*-aconitic acid.

**Biomass normalisation**

To facilitate the interpretation of treatment effects among genotypes, the amount of dry biomass produced was normalised according to:

\[
\frac{\text{Average biomass (AlPO}_4, \text{FePO}_4 \text{ or Ca}_5\text{OH(PO}_4)_3) - \text{Average biomass (0 P)}}{\text{Average maximum biomass (KH}_2\text{PO}_4) - \text{Average biomass (0 P)}}
\]

Thus, normalised biomass generally varied between 0 (biomass accumulated in the 0 P treatment, using only P present in the seed) and 1 (biomass accumulated in the KH\textsubscript{2}PO\textsubscript{4} treatment, at an adequate supply of P).

**Statistics**

Data were compared using one- and two-way ANOVA in GENSTAT 7.1, 7\textsuperscript{th} edn (Lawes Agricultural Trust 2003), with log transformations of the data, if required, followed by a multiple comparison Tukey test. Means are presented with standard errors.
Results

Total dry biomass varied among genotypes when P was supplied in the form of KH$_2$PO$_4$ (Figure 1a). Most landraces produced more biomass when P was supplied as Ca$_5$OH(PO$_4$)$_3$ than when provided with FePO$_4$ or AlPO$_4$, and equally well as when supplied with KH$_2$PO$_4$ for Kiev, GRC(5.4), KEN(5.7), MOR(7), GRC(7) and EGY(9) (Figure 1a; for accession codes see Table 1). The different response of landraces to different P sources is highlighted when the biomass is normalised (Figure 1b). AlPO$_4$ supply resulted in either less biomass than produced when supplied with the other sparingly soluble forms, or biomass production was equal to that which resulted from FePO$_4$ supply.

Leaf P concentrations were highest when P was supplied as KH$_2$PO$_4$, and similar for all genotypes, averaging 2.3 mg P g$^{-1}$ leaf dry mass (Figure 2). When P was supplied in the sparingly soluble forms, leaf P concentrations for all plants decreased to a similar extent, ranging from 0.8-1.4 mg P g$^{-1}$ leaf dry mass. There was no apparent link between leaf P concentration and form of sparingly soluble P supplied, despite difference in growth.

Differences in leaf biomass mirrored differences in total biomass (data not shown); senescence of leaves was accelerated in the 0 P treatment (Figure 3a). When no P was provided, some genotypes, e.g., MOR(7), GRC(7) and EGY(9), exhibited a much smaller percentage of senesced leaves (21-25 %) compared with others, e.g., Kiev, GRC(5.4), ETH(>7) or KEN(5.7), which shed 61-74 % of their leaves. MOR(7), GRC(7) and EGY(9) were also the best-performing landraces when P was supplied as AlPO$_4$ or FePO$_4$, producing the most biomass relative to KH$_2$PO$_4$ supply (Figure 1b).
While sparingly available P, compared with a supply of KH$_2$PO$_4$, did not lead to increased biomass allocation to roots, source of P did affect the proportion of roots invested in cluster roots. Dry biomass allocation to roots was different only for EGY(9), having a slightly greater root mass ratio (c. 0.26) than other landraces (c. 0.20). Root mass ratio did not vary significantly as a response to form of P supply within genotypes (data not shown). Percentage biomass allocation to cluster roots (dry mass basis) decreased for all landraces when P was supplied as KH$_2$PO$_4$, when it was reduced by 15-34% compared with the other treatments (Figure 3b). On average, the L. albus landraces allocated 42% of their root biomass to cluster roots when P was supplied in the sparingly soluble forms. Nodulation was not observed on any of the harvested root systems. 

Rhizosphere extract pH was approximately 1 pH unit higher when P was supplied in the form of KH$_2$PO$_4$, tending to be around pH 7, than when it was supplied as AlPO$_4$, FePO$_4$ or Ca$_5$OH(PO$_4$)$_3$, when the pH was between 5-6 (Figure 4). The bulk pH of the sand was 6.7 (Table 2).

Carboxylate concentrations in the rhizosphere were highly variable with 0 P or P supplied in sparingly soluble forms (Figure 5). Supply of P in the form of KH$_2$PO$_4$ reduced the accumulation of rhizosphere carboxylates for all landraces, except the cultivar Kiev mutant and KEN(5.7). The composition of carboxylates was similar for all plants and only changed when P was supplied as KH$_2$PO$_4$ which resulted in an increased proportion of malate from approximately 50 to 80% and corresponding decreased proportion of citrate. No other carboxylates were detected in appreciable amounts.
Discussion

Biomass response of accessions to P supply

The *L. albus* landraces and Kiev produced more biomass when P was supplied as Ca$_5$OH(PO$_4$)$_3$ than with AlPO$_4$ or FePO$_4$, with the exception of ETH(>7), which performed poorly with all P sources. The reason why most landraces were able to access Ca$_5$OH(PO$_4$)$_3$ better than the other poorly soluble forms relates to the greater dissolution of this P source at decreased pH which contrasts with the reduced availability of Al- and Fe-bound P at low pH (Lindsay 1979). As shown in Figure 4, the rhizosphere pH decreased when P was supplied as AlPO$_4$, FePO$_4$ or Ca$_5$OH(PO$_4$)$_3$, compared with the bulk sand pH of 6.7, favouring dissolution of Ca$_5$OH(PO$_4$)$_3$. Egyptian genotypes are reputed for being tolerant of calcareous soils (Raza *et al*. 2001; Kerley *et al*. 2002; Kerley and Huyghe 2002), and when P was supplied in the form of Ca$_5$OH(PO$_4$)$_3$, EGY(9) had the highest leaf [P] concentration (1.38 mg g$^{-1}$ leaf dry mass) followed by GRC(7) and PRT(5) (1.18 and 1.17 mg g$^{-1}$ leaf dry mass, respectively) (Figure 2). This may explain its success in calcareous sands.

Leaf P concentration did not change with form of sparingly soluble P supplied, despite difference in growth for some landraces compared with 0 P supply (Figures 1a, 2). P is mostly associated with a cell’s functional and structural components (nucleic acids, phospholipids; Mengel and Kirkby 2001) and a much smaller proportion is associated with the plant’s energy metabolism (Marschner 1995; Mengel and Kirkby 2001). In this study P was limiting due to its supply as sparingly soluble forms. The reason that leaf P concentrations did not increase, despite increases in growth, is likely to be that P taken up by the plant would be quickly incorporated into structural cell and
other functional components (structural proteins, nucleic acids) for growth, because very little P is required for the photosynthetic machinery required to achieve a greater productivity (De Groot et al. 2003).

Senescence of leaves, presumably related with P deficiency, was only elevated when no P was supplied, and for ETH(>7), which showed a high proportion of senesced leaves when P was supplied as AlPO₄, a sign of how poor this landrace was at accessing that form. Senescence was similarly low for sparingly soluble sources of P and for KH₂PO₄, suggesting that this is the normal developmental response of the plants, and that the supply of P in sparingly soluble forms did not induce a stress response due to either P deficiency or metal toxicity.

The substrate for growth in this study, river sand, has a low buffer capacity for pH. As a consequence, root-induced changes of rhizosphere pH may be greater in this sand than in some agricultural soils, and care must be taken with extrapolation of our results to field conditions. On the other hand, exudation and acidification are higher in the rhizosphere of clusters compared to non-clusters, so determinations on entire root systems are likely to underestimate localised pH decrease. Moreover, if these plants were grown under field conditions at low N supply, nodule formation and N₂ fixation might occur which would result in rhizosphere acidification (Raven et al. 1990). Rhizosphere acidification would influence pH interactions and, consequently, P solubilisation might have been different. In this study we supplied high levels of nitrate to the plants, thereby effectively suppressing nodulation.

Root mass ratio and cluster-root development
The unresponsiveness of the root mass ratio to form of P supply or absence of P, observed for all *L. albus* genotypes and forms of P, and despite increased shoot P status when P was supplied as KH$_2$PO$_4$, agrees with the finding for other *Lupinus* species (Lambers *et al*. 2006; Pearse *et al*. 2006b). There was no significant difference among genotypes in root mass ratio, suggesting there is little or no variation within the species for this trait. The percentage of the root systems that was invested in cluster roots did not vary among genotypes either, again suggesting little or no genetic variation; so, this is not a trait either that can readily be modified by selection within the species. Cluster-root production was significantly suppressed only with supply of P in the form of KH$_2$PO$_4$ for all genotypes except EGY(9), as expected (Keerthisinghe *et al*. 1998; Pearse *et al*. 2006a). While genotypic differences did not exist in the cluster-root proportion of root systems, differences may exist among genotypes in root architecture, which was not measured in this study, and has important implications for P-uptake (Lynch 1995).

*Rhizosphere carboxylate accumulation*

There was no significant variation among the genotypes in accumulated rhizosphere carboxylates when P was supplied as sparingly soluble forms. This is therefore not a key trait when selecting for P-acquisition-efficient genotypes. Accumulated rhizosphere carboxylate concentrations decreased for some genotypes only when P was supplied in the form of KH$_2$PO$_4$ which is not surprising given that it was the only form of P supplied that increased the P status of the plants and reduced the allocation of biomass to cluster roots. The change in carboxylate composition was correlated with variation in leaf P status, i.e. an increase in the percentage of malate relative to citrate at higher leaf [P]. The
shift in carboxylate composition when P was supplied in the soluble form of KH$_2$PO$_4$ has been reported for a number of other species (Pearse et al. 2006b), and the proportion of malate tends to increase with increasing leaf P status (Pearse et al. 2006a). In this study oxalate was not measured; in previous studies on L. albus carboxylate exudation, only trace amounts of oxalate were detected (e.g., Neumann and Römheld 1999; Watt and Evans 1999).

**P acquisition and ecogeographic origin of L. albus accessions**

Variation in growth and P uptake in presence of poorly soluble P forms among landraces was indeed found, but, contrary to expectation, it was not related to soil type at the site where the accessions were collected. Therefore, the ecogeographic distribution of L. albus landraces relates to traits other than those involved in P acquisition from sparingly soluble forms as supplied in this study, e.g., variation in tolerance to calcareous soils (Raza et al. 2001; Kerley et al. 2002; Kerley and Huyghe 2002), differences in internal P-utilisation efficiency, phosphatase release and adaptations to other environmental stresses. In a similar comparison of Glycine max (L.) Merr. genotypes from 3 different geographic locations contrasting in soil pH, Tang et al. (2007) also found differences in growth to be unrelated to carboxylate release and rhizosphere pH, but related to early vigour, nodulation and seed P reserves which may also be factors partially responsible for variation among L. albus genotypes.

**Relationship between acidification and carboxylate release**
The formation of proteoid roots in *L. albus* coincides with acidification of the environment adjacent to them (Gardner et al. 1981; Gardner and Parbery 1982b). In this study, the rhizosphere pH decreased when P was supplied in sparingly soluble forms (Fig. 4) which resulted in cluster-root formation and carboxylate exudation. Carboxylates are released as carboxylic anions, via an anion channel (Ryan et al. 1997; Hinsinger 2001; Lambers et al. 2006). Carboxylate release does not invariably coincide with proton release (Shane and Lambers 2005). A range of Australian Proteaceae exuded carboxylates without concomitant proton release, instead releasing K⁺ (Roelofs et al. 2001). *L. albus* cluster roots do not only release protons to balance carboxylate exudation; they predominantly release a range of cations, including K⁺, Na⁺ and Mg⁺ (Zhu et al. 2005). Zhu et al. (2005), using pharmacological agents to modify proteoid root exudation, found that proton efflux by young, fully developed *L. albus* cluster roots was not strictly correlated with citrate exudation because other cations, including K⁺ and Na⁺, can serve as counter-ions; however, proton efflux is strongly coupled to malate release. When comparing the pH and accumulated carboxylates for whole root system rhizosphere extracts in the present study (Figure 6), there was a correlation between accumulation of citrate and rhizosphere acidification; however, such a correlation did not exist for malate. Zhu *et al.* (2005) suggest that H⁺-pumping activity may be a superior strategy to maintain charge balance than release of K⁺ or Na⁺, because K⁺ or Na⁺ accompanying citrate release have to be taken up before they can be released. The transport system for ion uptake is energised by plasma-membrane H⁺-ATPases pumping H⁺ out of cells creating an electrochemical potential gradient across the plasma membrane (Palmgren 2001). Therefore, we can conclude that while net proton release
may not strictly occur with citrate exudation from cluster roots when *L. albus* is experiencing P deficiency, the net whole root system effect of cluster-root formation, carboxylate exudation and nutrient uptake over time is a correlation between citrate exudation and proton efflux, because of net proton release associated with charge balancing from root clusters and other root regions.

**Concluding remarks**

There was variation among landraces in ability to use P from the sparingly soluble forms; however, contrary to our hypothesis, this variation did not relate to soil type of origin of the landraces, and cannot be attributed to carboxylate release, cluster-root development or other physiological traits as measured in this study. Further comparative studies on localised root regions in response to P limitation, rhizosphere pH and internal P-utilisation efficiency are required to better understand genotypic variation within *L. albus* in ability to take up P from sparingly soluble forms.

**Acknowledgements**

This research was part of an Australian Research Council (ARC) Strategic Partnerships with Industry – Research & Training (SPIRT) scheme funding a PhD project in collaboration with the Department of Agriculture and Food, Western Australia, and CSBP FutureFarm. Colin Smith from the Department of Agriculture and Food, Western Australia provided the seeds and accession selection advice, for which we are very
grateful. Thanks to Madeleine Wouterlood, Ben Croxford, Aleksander Moreno and Jarrad King for assisting with the harvest. This manuscript was partially completed whilst funded by a Japan Society for the Promotion of Science (JSPS) Postdoctoral Award at the Japan International Research Center for Agricultural Sciences (JIRCAS).
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Table 1. Characteristics of the collection sites for the *L. albus* accessions.

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<th>Accession #</th>
<th>Identifier</th>
<th>Subspecies</th>
<th>Breeding status</th>
<th>Country of origin</th>
<th>Soil pH</th>
<th>Soil description</th>
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<td>22602</td>
<td>Kiev</td>
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<td>Ukraine</td>
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<td>Not applicable</td>
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<td>Landrace</td>
<td>Portugal</td>
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<td>Dull orange coarse sand</td>
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<td>GRC(5.4)</td>
<td>var. <em>albus</em></td>
<td>Wild - introduced</td>
<td>Greece</td>
<td>5.4</td>
<td>Dull yellow-orange sandy loam</td>
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<tr>
<td>26795</td>
<td>KEN(5.7)</td>
<td>var. <em>albus</em></td>
<td>Landrace</td>
<td>Kenya</td>
<td>5.7</td>
<td>Red loam</td>
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<td>MOR(7)</td>
<td>var. <em>graecus</em></td>
<td>Landrace</td>
<td>Morocco</td>
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<td>Grey-brown sandy loam</td>
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<td>Egypt</td>
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<td>28549</td>
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Table 2. Properties of the washed river sand.

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<td>Texture</td>
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<td>pH (1:5 CaCl$_2$)</td>
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<tr>
<td>NO$_3$-N + NH$_4$-N (mg kg$^{-1}$)</td>
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<tr>
<td>Total P (mg kg$^{-1}$)</td>
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<tr>
<td>Phosphorus-retention index (ml P g$^{-1}$ soil)</td>
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<tr>
<td>Reactive Iron (mg kg$^{-1}$)</td>
<td>120</td>
</tr>
</tbody>
</table>

The river sand was analysed by CSBP FutureFarm analytical laboratories (Bibra Lake WA, Australia). pH 1:5 soil:0.01 M CaCl$_2$ solution, ammonium and nitrate nitrogen as described by Searle (1984). Total P and Phosphorus-Retention Index (PRI) according to Allen and Jeffery (1990). Reactive iron was measured according to Parfitt and Childs (1988).
Figure legends

**Fig. 1** (a) Total dry biomass of eight *Lupinus albus* genotypes supplied with 0 (□) or 40 mg P kg$^{-1}$ dry sand in the forms of AlPO$_4$ (■), FePO$_4$ (■), Ca$_5$OH(PO$_4$)$_3$ (▲) or KH$_2$PO$_4$ (■). Error bars indicate standard error (n = 4); columns not sharing the same letter indicate significant differences between treatments within genotypes according to the Tukey test (P≤0.05). Differences among genotypes were significant for all treatments (P≤0.05). (b) Normalised biomass, i.e. biomass in the AlPO$_4$, FePO$_4$ or Ca$_5$OH(PO$_4$)$_3$ treatments minus the biomass in the 0 mg P kg$^{-1}$ treatment expressed as a proportion of the biomass in the KH$_2$PO$_4$ treatment minus the biomass in the 0 mg P kg$^{-1}$ treatment. Two-way ANOVA revealed significant sgenotype by treatment interactions (P<0.05).

**Fig. 2** P concentration of the leaves of eight *Lupinus albus* genotypes supplied with 0 (□) or 40 mg P kg$^{-1}$ sand in the forms of AlPO$_4$ (■), FePO$_4$ (■), Ca$_5$OH(PO$_4$)$_3$ (▲) or KH$_2$PO$_4$ (■). Error bars indicate standard error (n = 4); columns not sharing the same letter indicate significant differences between treatments within genotypes according to the Tukey test (P≤0.05). Differences among genotypes were significant for 0 P supply only (P≤0.05). Two-way ANOVA revealed genotype by treatment interactions were not significant.

**Fig. 3** Percentage of leaves that senesced (a), and fraction (DM basis) of the root system allocated to cluster roots (b) of eight *Lupinus albus* genotypes supplied with 0 (□) or 40 mg P kg$^{-1}$ sand in the forms of AlPO$_4$ (■), FePO$_4$ (■), Ca$_5$OH(PO$_4$)$_3$ (▲) or KH$_2$PO$_4$ (■). Columns not sharing the same letter indicate significant differences within genotypes.
according to the Tukey test ($P \leq 0.05$). ns indicates no significant difference. Differences among genotypes for leaf senescence was significant for 0 P and AlPO$_4$ treatments, and for root clusters was significant was significant for FePO$_4$ and Ca$_5$OH(PO$_4$)$_3$ treatments ($P \leq 0.05$). Two-way ANOVA revealed significant genotype by treatment interaction for leaves that senesced ($P \leq 0.05$), but not cluster root allocation.

**Fig. 4** pH of rhizosphere extracts of eight *Lupinus albus* genotypes supplied with 0 (□) or 40 mg P kg$^{-1}$ sand in the forms of AlPO$_4$ (□), FePO$_4$ (■), Ca$_5$OH(PO$_4$)$_3$ (■) or KH$_2$PO$_4$ (■). Columns not sharing the same letter indicate significant differences within genotypes according to the Tukey test ($P \leq 0.05$). Two-way ANOVA revealed differences were not significant for genotype by treatment interactions.

**Fig. 5** Concentration of carboxylates in the rhizosphere of eight *Lupinus albus* genotypes supplied with 0 (□) or 40 mg P kg$^{-1}$ sand in the forms of AlPO$_4$ (□), FePO$_4$ (■), Ca$_5$OH(PO$_4$)$_3$ (■) or KH$_2$PO$_4$ (■). Control pots were also measured for pH. Columns not sharing the same letter indicate significant differences within genotypes according to the Tukey test ($P \leq 0.05$). Differences were significant among genotypes for the FePO$_4$ treatment only ($P \leq 0.05$). Two-way ANOVA revealed differences were not significant for genotype by treatment interactions.
Fig. 6 Accumulation of (a) malate or (b) citrate in the rhizosphere as related to the pH of rhizosphere extracts of *L. albus* genotypes when supplied with 0 P (○) or 40 mg P kg⁻¹ dry sand in the forms AlPO₄ (■), FePO₄ (□), Ca₃OH(PO₄)₃ (▲) or KH₂PO₄ (●).
Figure 1.
Figure 2.

<table>
<thead>
<tr>
<th>Country</th>
<th>P Concentration (mg g⁻¹ leaf dry mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kiev</td>
<td>a</td>
</tr>
<tr>
<td>PRT (5)</td>
<td>a</td>
</tr>
<tr>
<td>GRC (5.4)</td>
<td>a</td>
</tr>
<tr>
<td>KEN (5.7)</td>
<td>b</td>
</tr>
<tr>
<td>MOR (7)</td>
<td>a</td>
</tr>
<tr>
<td>GRC (7)</td>
<td>a</td>
</tr>
<tr>
<td>EGY (9)</td>
<td>a</td>
</tr>
<tr>
<td>ETH (&gt;7)</td>
<td>a</td>
</tr>
</tbody>
</table>

Note: The bars with different letters indicate significant differences in P concentration.
Figure 3.
Figure 4.
Carboxylates in the rhizosphere
(µmol g\(^{-1}\) root dry mass)

- Kiev
- PRT (5)
- GRC (5.4)
- KEN (5.7)
- MOR (7)
- GRC (7)
- EGY (9)
- ETH (>7)

Figure 5.
Figure 6.

(a) Malate

R² = 0.014

(b) Citrate

R² = 0.628

Carboxylates in the rhizosphere
(µmol g⁻¹ root dry mass)

Rhizosphere extract pH

Carboxylates in the rhizosphere
(µmol g⁻¹ root dry mass)