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Short-term supply of elevated phosphate alters the belowground carbon allocation costs and functions of lupin cluster roots and nodules

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SUMMARY

Lupins can rely on both cluster roots and nodules for P acquisition and biological nitrogen fixation (BNF) respectively. The resource allocation (C, N and P) between cluster roots and nodules has been largely understudied during P-deficient conditions. The aim of this investigation was therefore to determine the changes in resource allocation between these organs during fluctuations in P supply. *Lupinus albus* was cultivated in sand culture for 3 weeks, with either sufficient (2 mM high) or limiting (0.1 mM low) P supply. Although variation on P supply had no effect on the total biomass, there were significant differences in specialised below-ground organ allocation to cluster roots and nodule formation. Cluster root formation and the associated C-costs increased during low P supply, but at sufficient P-supply the construction and growth respiration costs of cluster roots declined along with their growth. In contrast to the cluster root decline at high P supply, there was an increase in nodule growth allocation and corresponding C-costs. However, this was not associated with an increase in BNF. Since cluster roots were able to increase P acquisition under low P conditions, this below-ground investment may also have benefited the P nutrition of nodules. These findings provide evidence that when lupins acquire N via BNF in their nodules, there may be a trade-off in resource allocation between cluster roots and nodules.

Introduction

Phosphate (P) is one of the most limiting mineral nutrients for plant growth (Plaxton and Carswell, 1999; Raghothama, 1999, 2000). Its availability to the plant is limited by various properties of the soil itself and is largely determined by solubilisation of P containing compounds and P diffusion rates in the soil (Silberbush and Barber, 1983). Phosphate readily chelates to metal cations, clay particles and organic soil material rendering it unavailable for plant uptake (Jungk et al., 1993; Richardson 1994; Abel et al., 2002; Vance et al., 2003). Soil P is also influenced by pH, ionic strength, adsorption and dissolution from these particles (Vance et al., 2003). Plants display great phenotypic plasticity in acquisition strategies for macro-nutrients such as N and P, and can respond to P deficiency by means of a suite of adaptations at the morphological and biochemical level (Keertsinghe et al., 1998; Vance et al., 2003; Lambers et al., 2006).

It is well established that plants preferentially allocate resources to increase below ground biomass and growth under P limitation (Cakmak, 1994; Raghothama, 1999; Vance et al., 2003; Lambers et al., 2006) Cluster or proteoid roots are a combined physiological and morphological below-ground adaptation for phosphate (P) acquisition in P-deficient soils (Dinkelaker et al., 1995). The production of both cluster roots and roots will however incur a C and nutrient (N and P) cost to the plant. The root system alone can consume 11–14% of fixed C to maintain functionality (Kaschuk et al., 2009). Under P-limitation, cluster roots can constitute more than 50% of the root system (Reddell et al., 1997; Lamont and Structure, 2003). The exact costs of cluster roots vs. roots in relation to respiratory costs are currently unknown (Lamont and Structure, 2003; Lambers et al., 2006). Most species of plants associated with cluster root formation can also symbiotically fix atmospheric N_2 via biological nitrogen fixation (BNF) (Skene, 1998), but interestingly do not form mycorrhizal associations (Skene, 1998; Neumann and Martinoia, 2002).

It is known that nodulated plants expend more P on BNF when compared to direct N uptake mechanisms (Sa and Israel, 1991). Nodules act as strong sinks for P even during adequate P

Abbreviations: BNF, biological nitrogen fixation; PNUE, photosynthetic nitrogen use efficiency; PPUE, photosynthetic phosphate use efficiency; %NDFA, nitrogen derived from atmosphere; RGR, relative growth rates; SNAR, specific nitrogen acquisition rate; SNUR, specific nitrogen utilisation rate; SPAR, specific phosphate acquisition rate; SPUR, specific phosphate utilisation rate; PEPC, phosphoenolpyruvate carboxylase; PK, pyruvate kinase; NADH-MDH, malate dehydrogenase; ME, malic enzyme; APase, acid phosphatase.

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supply (Drevon and Hartwig, 1997). This trend is compounded during P deficiency where nodules often exhibit higher P content when compared to roots and shoots (Drevon and Hartwig, 1997). Hogh-Jensen et al. (2002) showed that P is preferentially partitioned to nodules for maintenance of BNF rates under P-deficiency, sometimes at the expense of plant growth. Apart from a strong P sink, nodules must also be supplied with photosynthate in the form of malate. Nodules thus incur a large C and P burden on the plant. The model legume, white lupin (*Lupinus albus*) readily nodulates with *Bradyrhizobium* sp. to form effective nodules (Schulze et al., 2006) and is also one of the best documented, cluster root forming species (Watt and Evans, 1999a,b; Neumann et al., 2002; Neumann and Martinoia, 2002; Lamont and Structure, 2003; Cheng et al., 2011). It is therefore an ideal model to use for the investigation of the costs associated with nutrient acquisition via nodules and cluster roots.

Overall, very little known about the costs of combined cluster roots and nodules under P deficiency in any lupin species. Therefore, the aim of this study was to investigate the below-ground allocation of C, N and P to nutrient acquisition organs (roots, nodules and cluster roots), during P deficiency in the model legume *L. albus*. In this regard, the carbon costs of both cluster roots and nodule development during P limitation was assessed, via biomass and growth kinetics, nutrient acquisition efficiencies, respiratory and photosynthetic costs.

Materials and methods

Plant growth conditions

L. albus (*Lupinus albus* cv. Andromeda) seeds were germinated in vermiculite before transplantation to sand culture. Seeds were sterilised and then inoculated with a commercially available inoculum (StimuPlant cc) containing *Bradyrhizobium* sp. (*Lupinus*) and grown in vermiculite for 10 days. Thereafter, plants were transplanted into 20 cm pots and cultivated in quartz sand for 30 days and received a modified Long Ashton solution (Smith et al., 1983) containing 0.1 mM $NaH_2PO_4{\cdot}2H_2O$ (control) as P source. After the 30 day growth period half of the treatment group was supplied with a high P Long Ashton solution (Smith et al., 1983) containing 2 mM $NaH_2PO_4{\cdot}2H_2O$ (control + HP) as P source (Keertsinghe et al., 1998; Le Roux et al., 2006, 2008) for 14 days, while the rest of the plants continued receiving the modified Long Ashton solution (Smith et al., 1983) containing 0.1 mM $NaH_2PO_4{\cdot}2H_2O$ (control) as P source. The pH of the solution was adjusted to 6.5, and 400 ml was applied to the plants once a week, furthermore, the plants received distilled H_2O every other day. No N source was added to ensure nodulation and BNF. Plants were grown under glasshouse conditions in a north-facing glasshouse at the University of Stellenbosch between the months of April and June. The range of midday irradiances was between 400 and 600 $\mu mol\ m^{-2}\ s^{-1}$ and the average night/day temperatures were 13–22 °C.

Harvesting and nutrient analysis

Seedlings were harvested 6 weeks after transplantation into the sand culture. Upon harvesting, the plants were separated into nodules, roots, stems and leaves. The harvested plant material was placed in a drying oven, at 40 °C for 3 days and their dry weights (DW) recorded. The dried material was milled with a ball mill. The milled samples were analysed for their respective C, N and P concentrations by a commercial laboratory, using inductive coupled mass spectrometry (ICP-MS) and a LECO-nitrogen analyser with suitable standards (BemLab, De Beers Road, Somerset West, SA.).

Cost and efficiency calculations

Construction costs, C_w (mmol C/g DW), were calculated according to Mortimer et al. (2005), modified from the equation used by Peng et al. (1993):

$$C_w = \left[\frac{C + k\mathrm{N}14 \times 180}{24}\right]\left(\frac{1}{0.89}\right)\left(\frac{6000}{180}\right)$$

where C_w is the construction cost of the tissue (mmol C/g DW), C is the carbon concentration (mmol C/g), k is the reduction state of the N substrate ($k = -3$ for NH_3) and N is the organic nitrogen content of the tissue (g/g DW) (Williams et al., 1987). The constant (1/0.89) represents the fraction of the construction cost which provides reductant that is not incorporated into biomass (Williams et al., 1987; Peng et al., 1993) and (6000/180) converts units of g glucose/g DW to mmol C/g DW.

Growth respiration, Rg(t) ($\mu mol\ CO_2$/day), is the daily growth respiration for the plant (Peng et al., 1993):

$$\mathrm{Rg}(t) = C_t - \Delta W_c$$

where C_t ($\mu mol\ CO_2$/day) is the C required for daily construction of new tissue. C_t was calculated by multiplying the root growth rate (gDW/day) by tissue construction cost (C_w). ΔW_c (μmol C/day) is the change in root C content and was calculated by multiplying the root C content and the root growth rate.

Specific P utilisation rate (SPUR) (g DW/mg P/d) is a measure of the DW gained for the P taken up by the plant (Mortimer et al., 2008):

$$\mathrm{SPUR} = \left[\frac{W_2 - W_1}{t_2 - t_1}\right] \times \left[\frac{\log eM_2 - \log eM_1}{M_2 - M_1}\right]$$

where M is the P content of the plant and W is the plant DW.

The specific nitrogen utilisation rate (SNUR) was adapted from the above equations to include N instead of P.

Belowground allocation represents the fraction of new biomass partitioned into new roots and nodules over the given growth period. This was calculated according to Bazzaz (1997):

$$\frac{df}{dt} = \mathrm{RGR}\left(\frac{\partial - \mathrm{Br}}{\mathrm{Bt}}\right)$$

where RGR is the relative growth rate (mg/g/day) and ∂ is the fraction of new biomass gained during the growth period. Br/Bt is the root weight ratio, based on total plant biomass (Bt) and root biomass (Br).

Calculations of $\delta^{15}N$

The δ^{15}N analyses were carried out at the Archeometry Department, University of Cape Town. The isotopic ratio of δ^{15}N was calculated as $\delta = 1000‰\ (R_{sample}/R_{standard})$, where R is the molar ratio of the heavier to the lighter isotope of the samples and standards is as defined by Farquhar et al. (1989). Between 2.100 and 2.200 mg of each milled sample were weighed into 8 mm × 5 mm tin capsules (Elemental Micro-analysis Ltd., Devon, UK) on a Sartorius microbalance (Goettingen, Germany). The samples were then combusted in a Fisons NA 1500 (Series 2) CHN analyser (Fisons instruments SpA, Milan, Italy). The δ^{15}N values for the nitrogen gas released were determined on a Finnigan Matt 252 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany), which was connected to a CHN analyser by a Finnigan MAT Conflo control unit. Three standards were used to correct the samples for machine drift: two in-house standards (Merck Gel and Nasturtium) and the IAEA (International Atomic Energy Agency) standard $(NH_4)_2SO_4$.