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Phosphorus deficiency affects the allocation of below-ground resources to combined cluster roots and nodules in *Lupinus albus*

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ABSTRACT

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). Slow soil diffusion rates and fast root uptake transporters, causes a rapid depletion of P in the rhizosphere, leading to irregular P distribution in the soil (Lambers et al., 2006). Organic and inorganic compounds readily interact and bind to P (Raghothama, 1999). 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 (Keerthisinghe 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. This is often at the expense of growth and photosynthesis (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 cluster roots will incur a C and nutrient (N and P) cost to the plant. Moreover, the cost of cluster root production must be kept to a minimum to decrease negative growth effects at the whole plant level. The root system alone can consume 11–14% of fixed carbon 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, 2003). P must ultimately be transported from the cluster roots to other plant organs if plant P status is to be maintained. It was shown by Keerthisinghe et al. (2002) that plant growth could be maintained, if cluster roots constitute more than 50% of the root system. The exact costs of cluster roots *vs.* roots in relation to respiratory costs are currently unknown (Lamont, 2003); however, cluster root growth and

Abbreviations: P Max, maximum rate of photosynthesis; 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.

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function must incur a large C burden on the plant (Lambers et al., 2006). Most species of plants associated with cluster root formation can symbiotically fix atmospheric nitrogen via biological nitrogen fixation (BNF) (Skene, 1998), but interestingly do not form mycorrhizal associations (Skene, 1998; Neumann and Martinoia, 2002).

Legumes are well known for their symbiotic relationship with rhizobia (Valentine et al., 2011). This symbiosis allows for N acquisition through BNF, bypassing the need for direct N uptake. BNF is a notoriously energetically expensive process, consuming on average 20 ATP (including obligatory H_2 evolution), per reaction, for the production of two NH_3 molecules (Schulze et al., 1999). Production of ATP is a high P requiring reaction, consuming phosphate per nitrogenase reaction. Comparatively, nitrate reduction to ammonia, after direct transporter uptake, indirectly consumes 15 ATPs (Valentine et al., 2011). It is furthermore, also 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 under adequate P supply (Drevon and Hartwig, 1997). This is compounded during P deficiency where nodules often exhibit higher P content when compared to roots and shoots (Drevon and Hartwig, 1997). Høgh-Jensen et al. (2002) also 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, 1999; Neumann et al., 2000; Neumann and Martinoia, 2002; Lamont, 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, there is 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

Lupinus albus (*L. 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 21 days. The plants were divided into two treatment groups, low phosphate (LP) and high phosphate (HP), each receiving a modified Long Ashton (Smith et al., 1983) solution containing either 2 mM (HP) or 0.1 mM (LP) $NaH_2PO_4{\cdot}2H_2O$ as phosphate source (Keerthisinghe et al., 1998; Le Roux et al., 2006, 2009). 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.

Photosynthesis and gas exchange measurements

The youngest fully expanded leaf was used for photosynthetic measurements. Light–response curves were used to determine the appropriate photon flux density (800 $\mu mol\,m^{-2}\,s^{-1}$) at which to conduct photosynthetic measurements. Readings were taken between 11 am and 4 pm, using the LI-6400XT portable photosynthesis and fluorescence system (Li-Cor, Lincoln, Nebraska,USA).

Photosynthetic CO_2 response curves were carried out in order to determine the maximum photosynthesis rate (Pmax), Rubisco activity and electron transport. Measurements were performed on the youngest fully expanded leaves (5 replicates in each treatment per species), using a Li-6400 gas exchange system (LI-COR Inc., IRGA, Lincolin, NE, USA). Measurements were taken between 9 am and 4 pm. A full response curve took 45 min to 1 h to complete. The leaves were enclosed in a leaf chamber (6 cm^2), which received a steady light of 800 $\mu mol\,m^{-2}\,s^{-1}$ at a leaf temperature of 24 °C. CO_2 concentrations increased according to the following increments: 50 and 100 ppm.

Harvesting and nutrient analysis

Seedlings were harvested at 30 days 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) were 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 inductively coupled mass spectrometry (ICP-MS) and a LECO-nitrogen analyser with suitable standards (BemLab, De Beers Road, Somerset West, SA).

Carbon and nutrition cost calculations

Construction costs, C_W ($mmol\,C\,g^{-1}\,DW$), were calculated according to the methods of Mortimer et al. (2005), modified from the equation used by Peng et al. (1993):

$$C_W = \left[C + kN \times \frac{180}{24}\right]\left(\frac{1}{0.89}\right)\left(\frac{6000}{180}\right)$$

where C_W is the construction cost of the tissue ($mmol\,C\,g^{-1}\,DW$), 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/DW) (Williams et al., 1987). The constant (1/0.89) represents the fraction of the construction costs that provides reductant that is not incorporated into the biomass (Williams et al., 1987; Peng et al., 1993) and (6000/180) converts units of g glucose/DW to mmol C g/DW.

Specific nitrogen absorption rate (SNAR) ($mg\,N\,g^{-1}\,root\,DW\,d^{-1}$) is the calculation of the net N absorption rate per unit root DW (Nielson et al., 2001):

$$SNAR = \left[\frac{M_2 - M_1}{t_2 - t_1}\right] \times \left[\frac{\log_e R_2 - \log_e R_1}{R_2 - R_1}\right]$$

where M is the N content per plant, t is the time and R is the root DW.

Specific nitrogen utilisation rate (SNUR) ($g\,DW\,mg^{-1}\,N\,d^{-1}$) is a measure of the DW gained for the N taken up by the plant (Nielson et al., 2001): $SNUR = \left[\frac{W_2 - W_1}{t_2 - t_1}\right] \times \left[\frac{\log_e M_2 - \log_e M_1}{M_2 - M_1}\right]$

Specific P absorption rate (SPAR) ($mg\,N^{-1}\,g\,root\,DW^{-1}\,d^{-1}$) is the calculation of the net P absorption rate per unit root DW (Nielson et al., 2001): $SPAR = \left[\frac{M_2 - M_1}{t_2 - t_1}\right] \times \left[\frac{\log_e R_2 - \log_e R_1}{R_2 - R_1}\right]$ where M is the P content per plant, t is the time and R is the root DW.