

Effects of phosphorus supply on growth, phosphate concentration and cluster-root formation in three *Lupinus* species

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- **Background and Aims** In some lupin species, phosphate deficiency induces cluster-root formation, which enhances P uptake by increasing root surface area and, more importantly, the release of root exudates which enhances P availability.
- **Methods** Three species of *Lupinus*, *L. albus*, *L. atlanticus* and *L. micranthus*, with inherently different relative growth rates were cultivated under hydroponics in a greenhouse at four phosphate concentrations (1, 10, 50 and 150 μM) to compare the role of internal P in regulating cluster-root formation.
- **Key Results** The highest growth rate was observed in *L. atlanticus*, followed by *L. albus* and *L. micranthus*. At 1 μM P, cluster-root formation was markedly induced in all three species. The highest P uptake and accumulation was observed in *L. micranthus*, followed by *L. atlanticus* and then *L. albus*. Inhibition of cluster-root formation was severe at 10 μM P in *L. atlanticus*, but occurred stepwise with increasing P concentration in the root medium in *L. albus*.
- **Conclusions** In *L. atlanticus* and *L. albus* cluster-root formation was suppressed by P treatments above 10 μM , indicating a P-inducible regulating system for cluster-root formation, as expected. By contrast, production of cluster roots in *L. micranthus*, in spite of a high internal P concentration, indicated a lower sensitivity to P status, which allowed P-toxicity symptoms to develop.

Key words: Cluster roots, lupin, *Lupinus*, phosphate nutrition, toxicity, uptake.

INTRODUCTION

Phosphorus (P) is an essential macronutrient for plant growth, and it is limiting crop production in many regions of the world (Holford, 1997). Global demand for P fertilizer continues to increase while global reserves of P are in decline (Steen, 1998; Cordell *et al.*, 2009). The availability of soil P for plants is related to several plant characters, including the release of carboxylates (Ryan *et al.*, 2001), rhizosphere pH (Curtin *et al.*, 1993; Hinsinger, 2001), morphological traits such as length and surface area of roots (Williamson *et al.*, 2001; Li *et al.*, 2007), root architecture (Lynch, 1995), root hairs (Gahoonia and Nielsen, 1997), mycorrhizas (Smith *et al.*, 2000) and specialized structures such as root clusters (Lambers *et al.*, 2003; Shane and Lambers, 2005). Several investigators have reported that P is a key element that strongly influences the initiation and growth of cluster roots (Shane and Lambers, 2005). In field studies, some grain legumes exhibit higher P-acquisition efficiency than other crops due to cluster-root formation and release of carboxylate (Bolland *et al.*, 1999).

Cluster roots release large quantities of carboxylates in an exudative burst from mature clusters (Watt and Evans, 1999; Shane and Lambers, 2005). On the other hand, adequate P supply to plants inhibits cluster-root formation and so avoids excessive loss of carbon from the root system (Keerthisinghe *et al.*, 1998). Several investigators have reported either initiation or inhibition of cluster-root formation to be linked

to internal P concentration in the plant (Shane *et al.*, 2003; Li and Liang, 2005; Shen *et al.*, 2005). However, the exact location of the signal has not yet been fully elucidated; it might be the shoot P concentration, the P concentration in phloem sap or the P concentration in the root (Shane *et al.*, 2003). Recently, Pearse *et al.* (2006) introduced a model that cluster roots in *Lupinus* species have a positive effect on P uptake, which enhances P status, but this, in turn, has a negative effect on cluster-root formation and exudation. The P concentration in a plant is balanced by phosphate uptake and plant growth rate. The capacity for P uptake may be affected by several environmentally or genetically controlled factors that differ among *Lupinus* species (Pearse *et al.*, 2006). Also, a plant's growth rate may be influenced by many ecological or genetic factors. If shoot P concentration is a signal for prevention of cluster-root formation, different species with inherent differences in maximum growth rate and P uptake may differ in the foliar P concentration that either initiates or suppresses cluster-root formation. In this way, those plants that grow quickly, even with a higher uptake of phosphate, may accumulate little P in their shoot and stimulate cluster-root formation. By contrast, species that have an inherently low growth rate may accumulate high levels of P in their shoot, even at a relatively low phosphate supply. It is therefore of interest to evaluate changes in P concentration and cluster-root formation in different plants with inherently different growth rates as affected by phosphate supply in their root medium.

The main objective of the present study was to evaluate the effect of a plant's relative growth rate on its shoot P concentration and cluster-root formation. Based on preliminary experiments, three *Lupinus* species with inherently different growth rates, *L. albus*, *L. atlanticus* and *L. micranthus*, were grown in hydroponic culture at four P concentrations. These species are all Old World species, which naturally occur in the Mediterranean and North Africa (their origins are: *L. albus*, Egypt; *L. atlanticus*, Morocco; *L. micranthus*, Israel) (Käss and Wink, 1997). The P concentration in shoot and root and plant growth rate were determined to assess possible correlations with cluster-root formation.

MATERIALS AND METHODS

Seeds of *Lupinus albus* L. 'Kiev mutant', *L. micranthus* L. 'P22945' and *L. atlanticus* L. 'P27224' were scarified by scalpel and hydrated for 5 h. The hydrated seeds were sown in pots filled with washed river sand in a glasshouse on 31 May, 2007 and watered lightly daily. Seedlings were carefully removed and the roots washed free of sand on 8 June, 2007. Healthy seedlings of equal size for each species were selected for hydroponic culture. Each plant was supported in the centre of a grey foam lid, and the roots immersed in a 20-L black plastic container filled with continuously aerated nutrient solution of the following composition (μM): Ca(NO₃), 400; K₂SO₄, 200; MgSO₄, 54; MnSO₄, 0.24; ZnSO₄ 0.1; CuSO₄, 0.018; H₃BO₃, 2.4; Na₂MoO₄, 0.03; Fe-EDTA, 10. De-ionized water was used for making the nutrient solution and the entire solution was replaced daily. The pots were placed in a temperature-controlled root-cooling tank maintained at 18–22 °C. The experiment was carried out in a factorial, completely randomized design. Factor 1 was phosphate concentration in the root medium and factor 2 was species. The cotyledons were removed before starting treatments to limit plants to phosphate absorbed by roots. During the experimental period, the midday irradiance in the glasshouse was approx. 900 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, the day/night air temperature was 11/27 °C and the average relative humidity was 67 %.

The experiment from the day of sowing lasted 54 d. Four plants from each species and treatment were harvested randomly at 8, 32, 39, 47 and 54 d after sowing. There was one more harvest for *L. albus* at day 25 after sowing. Each plant was separated into stems (including petioles), leaves, cluster roots and non-cluster roots, and the components were weighed, dried in an oven for 3 d at 70 °C, re-weighed and ground for determination of P concentration. Leaves, cluster roots and non-cluster roots were scanned before drying (see below) and root mass ratio (root dry mass/total dry mass) was calculated. Relative growth rates (RGRs), based on total dry mass, were calculated using the following equation (Ricker, 1979)

$$\text{RGR} = \frac{(\ln M_2 - \ln M_1)}{T_2 - T_1}$$

where M_2 is the final dry mass (day 54), M_1 the initial dry mass (day 8) and T the number of days of growth.

Scanning and image analysis of roots and leaves

Each root sample (cluster roots and non-cluster roots) was dispersed in water in a transparent tray (30 × 20 × 3 cm), and scanned (EPSON Expression 1600, Seiko EPSON Corp., Nagano, Japan) with a resolution of 400 d.p.i. All leaflets of each sample were separated and spread in a transparent tray (without water) and scanned. Where there was a large amount of roots or leaves, up to four trays were used for scanning of one sample. The images were analysed with WinRHIZO software by the method of object separation from background and classification of pixel colours (Regent Instruments Inc., Sainte-Foy, Quebec, Canada). Leaf area, root length and root diameter distribution were measured and leaf/root area ratio ($\text{m}^2 \text{m}^{-2}$) was calculated.

Determination of total phosphate and phosphate uptake

Concentrations of total P were determined in roots and shoots after digesting approx. 100 mg of dried and powdered plant material in a mixture of concentrated nitric and perchloric acids (3 : 1) at 175 °C. Total P was assayed using a UV-vis spectrophotometer (Shimadzu Corp., Kyoto, Japan) by the malachite green oxalate method (Irving and McLaughlin, 1990).

P uptake rate [J , mg g^{-1} (root dry mass d^{-1})] was calculated from dry weights and P concentrations for each replicate and was averaged using the following equation (Soon, 1988):

$$J = \frac{(\ln M_2 - \ln M_1)}{T_2 - T_1} \times \frac{P_2 - P_1}{M_2 - M_1}$$

where J is the mean uptake rate (mg g^{-1} root dry mass d^{-1}), M is the root dry mass (g), P is the amount of P (mg g^{-1} dry mass) in the plant and T is time (d). For calculation of P uptake based on root area [mg m^{-2} (root area) day^{-1}], root area (m^2) was substituted for root mass in the equation above. The P-uptake rate was calculated for plants between days 39 and 54 after sowing.

Statistical analysis

All data were analysed by analysis of variance (ANOVA) using SAS statistical software (SAS Institute Inc., Cary, NC, USA). Comparisons of means were performed using Duncan's multiple range analysis test ($\alpha = 0.05$). Logarithmic curves were fitted using regression analysis to describe the relationship between plant investment in cluster roots and leaf, stem and root P concentration

RESULTS

Deficiency and toxicity symptoms

In both *L. albus* and *L. atlanticus*, P deficiency at 1 μM phosphate in the nutrient solution caused chlorosis, necrosis and marginal scorching of old leaves, which led to shedding of some of the old leaves (Fig. 1). There were no toxicity symptoms in *L. albus* and only moderate toxicity symptoms in *L. atlanticus*, even at 150 μM P. Conversely, in *L. micranthus* phosphate toxicity was visible at 10 μM P and became more

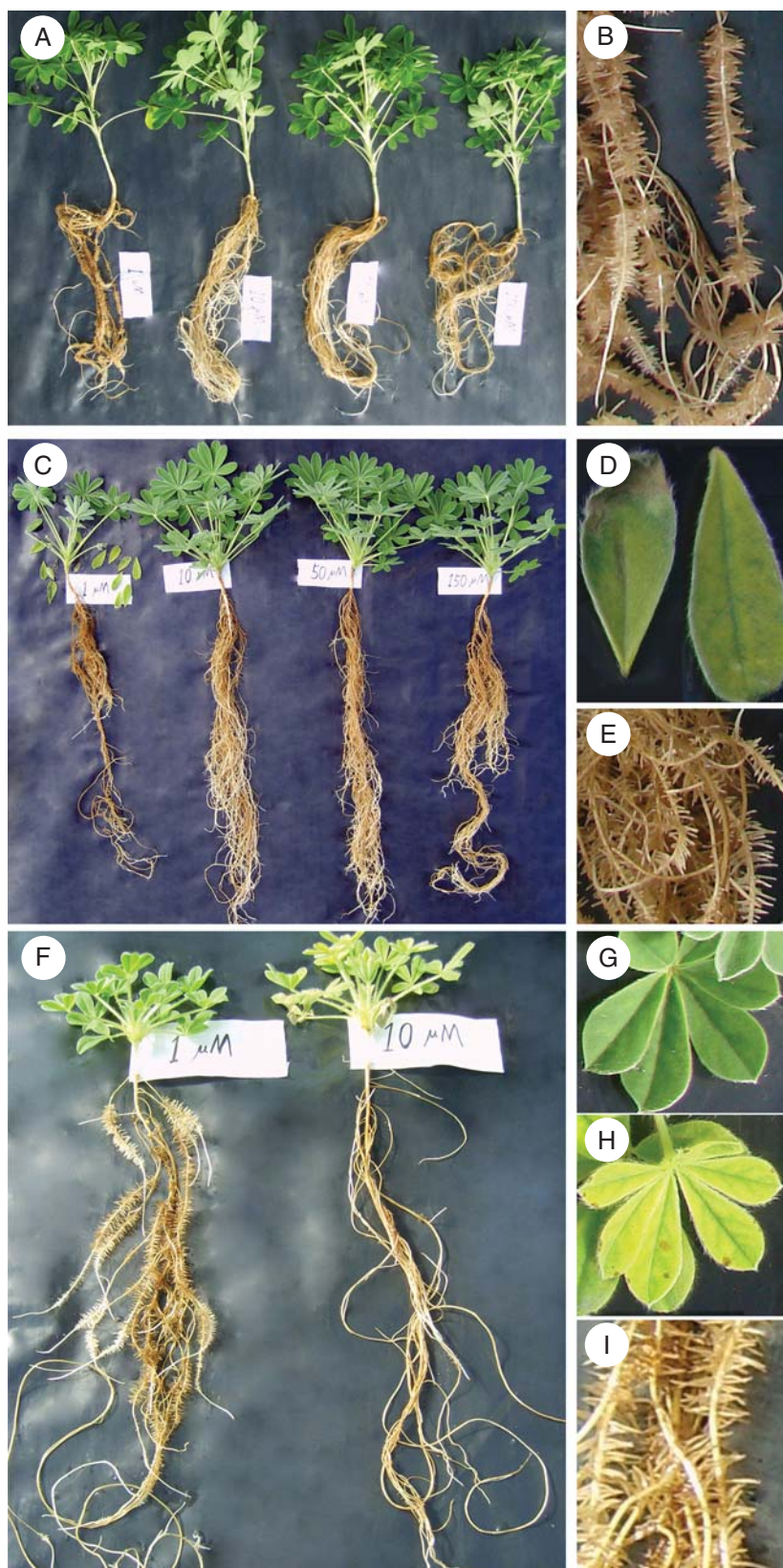


FIG. 1. Effect of phosphate concentration in the root environment on plant growth. (A) *Lupinus albus* whole plants, (B) *L. albus* cluster root of a plant grown at 1 μM P, (C) *L. atlanticus* whole plants, (D) *L. atlanticus* leaves showing P deficiency, (E) *L. atlanticus* cluster root of a plant grown at 1 μM P, (F) *L. micranthus* plants, (G) *L. micranthus* healthy leaf of a plant grown at 1 μM P, (H) *L. micranthus* leaf showing P toxicity when plants are grown at 10 μM P and (I) *L. micranthus* cluster root of a plant grown at 1 μM P.

pronounced at higher P concentrations. There were no deficiency symptoms, even at $1 \mu\text{M}$ P, in *L. micranthus*, whereas toxicity resulted in interveinal chlorosis and marginal necrosis, leading to shedding of old leaves and leaf desiccation at 50 and $150 \mu\text{M}$ P.

Growth

Based on total plant dry mass, the highest relative growth rate was observed in *L. atlanticus*, followed by *L. albus*, and the lowest growth rate was exhibited by *L. micranthus* (Figs 1 and 2, Table 1).

In *L. albus*, P deficiency, first visible after 32 d, caused more than 60% reduction of dry mass at $1 \mu\text{M}$ P compared with $50 \mu\text{M}$ P (Fig. 1). Dry mass was highest at $50 \mu\text{M}$ P and harvested on day 54 (Fig. 2). Similarly, root and leaf area were higher at $50 \mu\text{M}$ P and lower at $1 \mu\text{M}$ P, compared with other treatments at the final harvest (Fig. 3, Table 1).

In *L. atlanticus*, P deficiency caused a significant reduction in growth at $1 \mu\text{M}$ P after 32 d. Also, phosphate toxicity resulted in a marked reduction in growth at $150 \mu\text{M}$ P (Fig. 2). At the final harvest, maximum growth was observed at 10 and $50 \mu\text{M}$ P. Root and leaf area were higher at 10 and $50 \mu\text{M}$ P and lower at $1 \mu\text{M}$ P in comparison with other treatments at the final harvest (Fig. 3). Comparing the 50 and $150 \mu\text{M}$ P treatments, phosphate toxicity was more severe, showing more than 25% reduction in dry mass in *L. atlanticus* in comparison with less than 15% in *L. albus*.

L. micranthus was very sensitive to phosphate toxicity, showing significant reduction of fresh and dry mass at 50 and $150 \mu\text{M}$ P from the first harvest onwards (day 32), and subsequently (after 39 d of treatment) desiccation of the leaves (Fig. 1). The highest fresh and dry mass was observed at $1 \mu\text{M}$ P, and even $10 \mu\text{M}$ P caused mild toxicity (Figs 1 and 2). The same result was found for leaf area, where $1 \mu\text{M}$ P gave the highest leaf area (Fig. 3).

The leaf/root area ratio was higher in *L. albus* than in *L. atlanticus* and *L. micranthus*, and this ratio increased for all species with increasing P supply (Table 1).

The root mass ratio (root dry mass/total dry mass) varied significantly among species, and, for some species, between treatments. The mass of roots was significantly less in *L. albus* than in *L. atlanticus* and *L. micranthus*, on average representing 33, 28 and 26% of the whole plant on day 54, respectively. In *L. albus* and *L. micranthus*, but not in *L. atlanticus*, allocation of biomass to roots compared with whole plants increased significantly when supplied with the higher level of P (37% at $1 \mu\text{M}$ P to 32% at $10 \mu\text{M}$ P in *L. albus* and 33% at $1 \mu\text{M}$ P to 21% at $1 \mu\text{M}$ P in *L. micranthus*) (Table 1).

Cluster-root formation

The highest dry mass and area of cluster roots was exhibited at $1 \mu\text{M}$ P in all species; increasing P concentration in the root environment decreased cluster-root formation (Figs 1 and 3, Table 1). The most severe inhibition of cluster-root formation by P was observed in *L. atlanticus*, where the dry mass of cluster roots decreased from 38% of total root mass at $1 \mu\text{M}$ P to less than 1% at $150 \mu\text{M}$ P. The highest percentage of

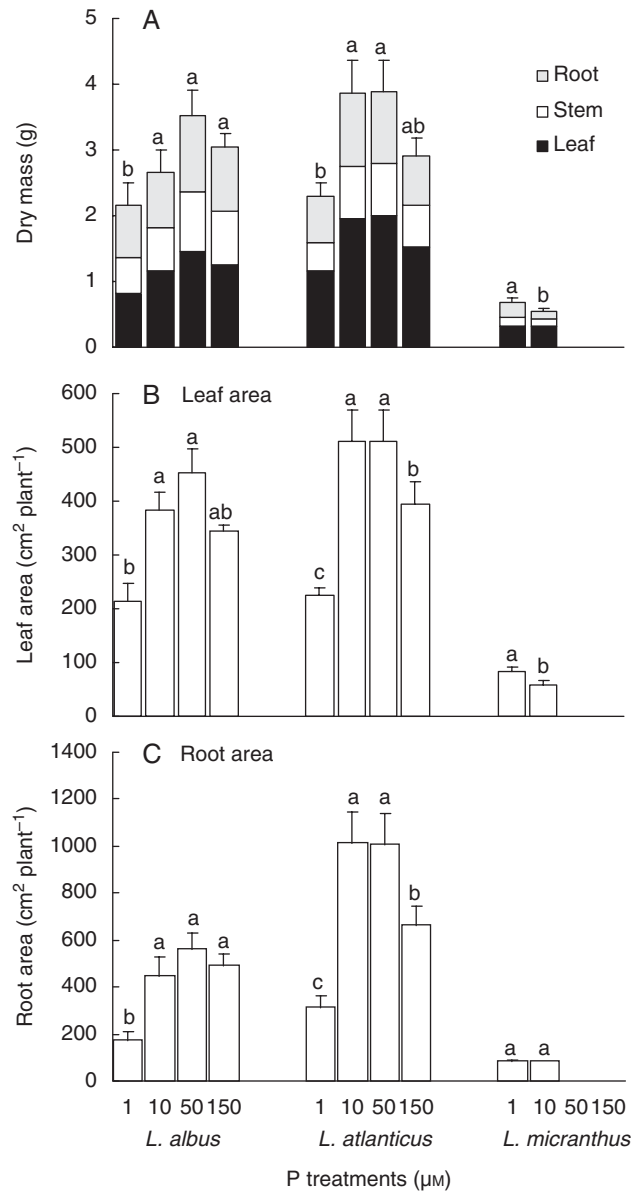


FIG. 2. Effects of phosphate concentration in the root environment on dry mass (A), leaf area (B) and root area (C) of *Lupinus albus*, *L. atlanticus* and *L. micranthus* plants after 54 d in hydroponics. Treatment means marked with the same lower-case letter are not significantly different within each group using a one-way ANOVA for each harvest followed by Duncan's multiple test ($P < 0.05$).

cluster roots was shown by *L. micranthus* followed by *L. albus* (Fig. 3). These differences coincided with the apparent rate at which clusters developed: on day 32, the generally slow-growing *L. micranthus* at $1 \mu\text{M}$ P already had 44% cluster roots, whereas the faster-growing *L. albus* and *L. atlanticus* had 21 and 15%, respectively.

Phosphate uptake and concentration

The P concentration in plant tissues was very low at $1 \mu\text{M}$ P, but increased gradually with increasing P concentration in the root environment (Fig. 4). The highest concentration of P was

TABLE 1. Effects of phosphate treatments on root mass ratio (total dry mass/root dry mass), leaf/root area ratio, relative growth rate and P uptake in *Lupinus albus*, *L. atlanticus* and *L. micranthus* plants during 54 d in hydroponics culture

P treatment (μM)	Root mass ratio, day 54 (g g^{-1})	Leaf/root area ratio, Day 54 ($\text{cm}^2 \text{cm}^{-2}$)	Relative growth rate, days 8/54 (d^{-1})	P uptake	
				$\text{mg g}^{-1} \text{d}^{-1}$	$\text{mg m}^{-2} \text{d}^{-1}$
<i>L. albus</i>					
1	0.37 ^a	0.46 ^b	0.039 ^b	0.06 ^c	0.83 ^c
10	0.32 ^b	0.70 ^a	0.044 ^{ab}	0.36 ^c	4.55 ^c
50	0.33 ^b	0.64 ^a	0.050 ^a	4.90 ^a	77.44 ^a
150	0.32 ^b	0.60 ^a	0.047 ^{ab}	3.35 ^b	49.72 ^b
Mean	0.33 ^a	0.60 ^a	0.045 ^b	2.45 ^b	33.14 ^b
<i>L. atlanticus</i>					
1	0.30 ^a	0.34 ^b	0.049 ^b	0.09 ^c	0.91 ^c
10	0.29 ^a	0.51 ^{ab}	0.061 ^a	2.43 ^b	25.43 ^b
50	0.28 ^a	0.51 ^{ab}	0.060 ^a	5.52 ^a	62.20 ^a
150	0.27 ^a	0.66 ^a	0.057 ^a	6.13 ^a	63.54 ^a
Mean	0.28 ^b	0.52 ^b	0.057 ^a	3.54 ^a	38.02 ^a
<i>L. micranthus</i>					
1	0.33 ^a	0.30 ^b	0.048 ^a	0.09 ^b	0.70 ^b
10	0.21 ^b	0.58 ^a	0.036 ^b	4.81 ^a	44.71 ^a
50					
150					
Mean	0.27	0.44	0.042	2.17	22.21

Treatments marked with the same lower-case letter are not significantly different within each species using one-way ANOVA followed by Duncan's multiple test ($P < 0.05$). Treatment means indicated a difference among species using two-way ANOVA followed by Duncan's multiple test ($P < 0.05$); not relevant for *L. micranthus* because there were no data for 50 and 150 μM P.

observed in *L. micranthus* (50 mg g^{-1} d. wt). The rate of P uptake increased at higher P concentrations in all three species. Comparing the three species at 10 μM P, the highest P-uptake rate was observed for *L. micranthus*, followed by *L. atlanticus* and *L. albus*.

Relationship between P concentration and cluster-root formation

Based on regression analysis, there was a significant correlation between cluster-root formation and P concentration in all species and for P concentration in all plant organs. The best curves for exhibition of these correlations were polynomial. Cluster-root formation decreased sharply as P concentrations in roots, stems and leaves of all three species increased (Fig. 5). The highest inhibition of cluster-root formation by P concentration in leaves was in *L. atlanticus*, where cluster-roots decreased to less than 1% by increasing P concentration of leaves to about 20 mg g^{-1} d. wt in comparison with 5 and 27% in *L. albus* and *L. micranthus*, respectively. The differences among species in cluster-root formation as affected by P concentration were even more obvious when comparing P concentrations in stems, showing a reduction of cluster-roots to 5% at 6, 21 and 43 mg P g^{-1} d. wt in *L. atlanticus*, *L. albus* and *L. micranthus*, respectively.

DISCUSSION

The main objective of the present study was to assess the effect of phosphate supply on cluster-root formation in three lupin species with different relative growth rates and to test the model proposed by Pearse *et al.* (2006). The results indicate major differences in growth and P status in response of the three *Lupinus* species to P supply. Relative growth rates

increased from *L. micranthus* to *L. albus* followed by *L. atlanticus* (Table 2). Conversely, the highest specific rate of P uptake (P uptake per unit root mass or area) was observed for *L. micranthus*, followed by *L. atlanticus* and *L. albus* (Fig. 4, Table 2). At 1 μM P in the root environment, all plants produced cluster roots, but cluster-root formation decreased with increasing rate of P concentration in the nutrient solution (Fig. 3), consistent with previous findings for *L. albus* (e.g. Keerthisinghe *et al.*, 1998; Watt and Evans, 1999) and *L. atlanticus* (Pearse *et al.*, 2006). Below, we provide critical insights into the differences in response among species.

Sensitivity to P supply differed among the present *Lupinus* species. At 1 μM P, on day 54 the concentration of P in leaves of *L. albus* and *L. atlanticus* was 1.68 and 0.93 mg g^{-1} d. wt, respectively which caused P-deficiency symptoms (Figs 1 and 4). In *L. micranthus*, on day 39 the concentration of P in leaves at 1 μM P was 3.62 mg g^{-1} d. wt with no symptoms of P deficiency; however, on day 54 the concentration of P in leaves was only 2.26 mg g^{-1} d. wt, above the critical leaf P concentration previously determined for *L. albus* (Bolland *et al.*, 1999). The P concentration on day 54 increased up to 19.9 and 21.6 mg g^{-1} d. wt in leaves of *L. albus* and *L. atlanticus*, respectively, at 150 μM P without any visual signs of toxicity such as chlorosis of leaves; however, root and leaf area and total dry mass decreased markedly compared with that at 50 μM P (Fig. 4). The P concentration of *L. micranthus* increased from 40.23 mg g^{-1} d. wt at 50 μM P to 43.7 mg g^{-1} d. wt at 150 μM P on day 39 with visible symptoms of P toxicity, leaf desiccation and drying of plants (Figs 1, 2 and 4). Development of foliar symptoms of phosphate toxicity, even at very low rhizosphere [P], has been reported in Proteaceae (e.g. in *Banksia ericifolia*, Ozanne and Specht, 1981; Parks *et al.*, 2000; *B. grandis*, Lambers *et al.*, 2002; *Hakea prostrata*, Shane *et al.*, 2004).

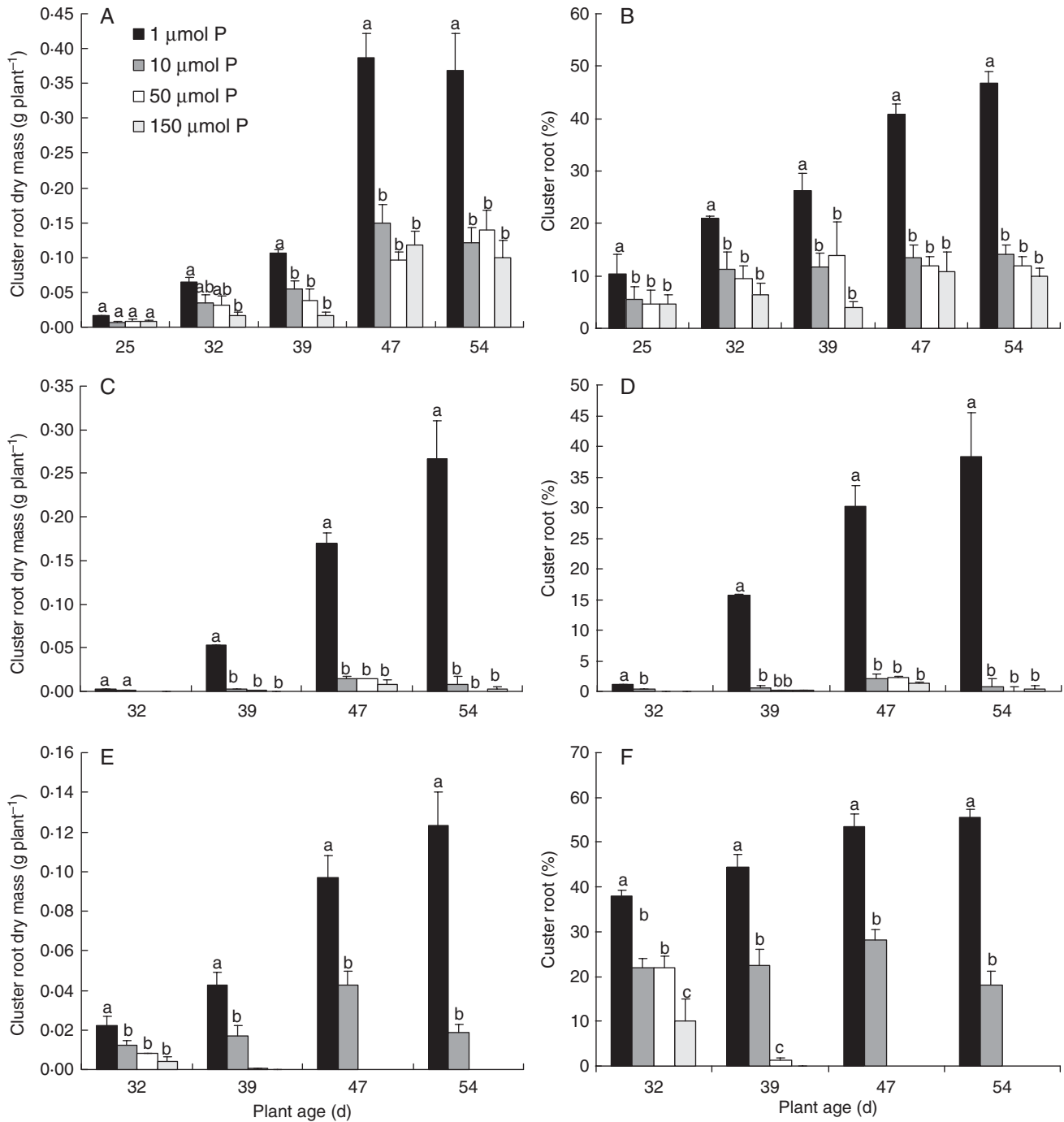


FIG. 3. Effects of phosphate concentration in the root environment on cluster-root dry mass and percentage of cluster roots as compared with total roots based on dry mass in *Lupinus* species during 54 d in hydroponics. *L. albus* (A, B), *L. atlanticus* (C, D) and *L. micranthus* (E, F). Treatment means marked with the same lower-case letter are not significantly different within each group using a one-way ANOVA for each harvest followed by Duncan's multiple test ($P < 0.05$).

The percentage cluster roots at the lowest P concentration (1 μM) decreased from *L. micranthus* to *L. albus*, followed by *L. atlanticus* (Fig. 3). In *L. micranthus*, greater cluster-root formation in this treatment from day 32 may have resulted in more efficient P uptake and accumulation, and consequently adequate P supply (Fig. 4, Tables 1 and 2). In *L. albus* and *L. atlanticus*, formation of cluster roots at 1 μM P during

growth was not as high as in *L. micranthus*; P concentration was not adequate and deficiency symptoms were shown (Figs 1, 3 and 4).

In *L. atlanticus*, induction of cluster-root formation was high at 1 μM P, but decreased drastically at higher concentrations when cluster-root dry weight decreased by 98% from 1 to 10 μM P (Fig. 3, Table 2). In spite of a high growth rate, the

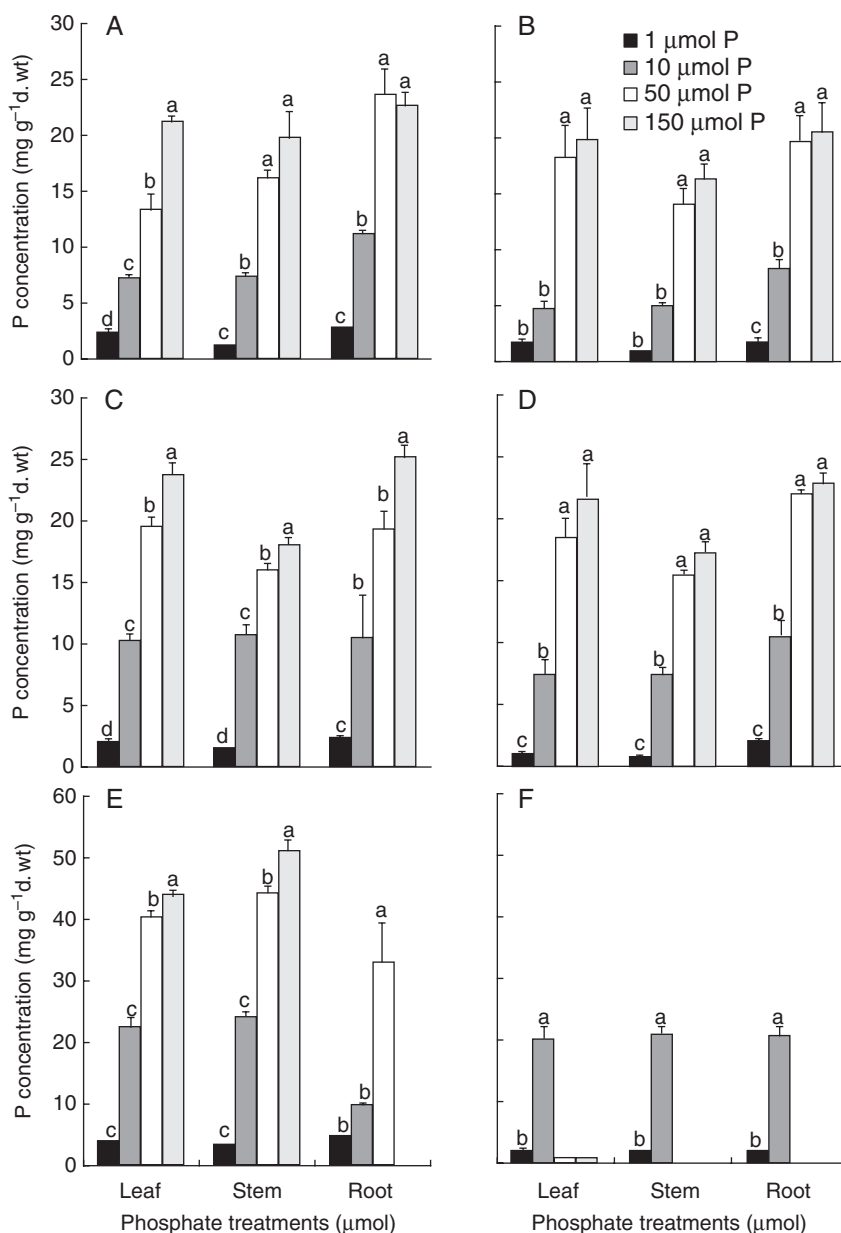


FIG. 4. Effects of phosphate concentration in the root environment on P concentration in *Lupinus* species during 54 d of hydroponics culture. *L. albus* day 39 (A), *L. albus* day 54 (B), *L. atlanticus* day 39 (C), *L. atlanticus* day 54 (D), *L. micranthus* day 39 (E), *L. micranthus* day 54 (F). Treatment means marked with the same lower-case letter are not significantly different within each group using a one-way ANOVA for each harvest followed by Duncan's multiple test ($P < 0.05$).

relatively high P-uptake rate resulted in intermediate accumulation of P in plants (Table 2, Fig. 4). As a result, cluster-root formation suppressed at 10 μM P (7–10 mg g^{-1} d. wt in leaves). Regression analysis also indicated that highest inhibition of cluster-root formation by P concentration was in *L. atlanticus* (Fig. 4). This species provides a good example for confirmation of the model proposed by Pearse *et al.* (2006), although the P treatments in the study by Pearse *et al.* (2006) were not low enough to see such a great change in proportion of cluster roots. Inhibition of cluster-root formation has been reported in *Banksia ericifolia* at >5 mg g^{-1} shoot d. wt P (Handreck, 1991) and *Banksia grandis* (Lambers *et al.*, 2002), whereas cluster roots were abundant

at <1 mg P g^{-1} d. wt. Cluster-root formation is drastically inhibited when P concentrations reach a threshold. However, different *Lupinus* species show a different threshold for inhibition of cluster-root formation.

In *L. albus*, with an intermediate growth rate and low P-uptake rate, cluster-root formation was extremely high at 1 μM P, and decreased stepwise at higher P concentrations (Table 1, Figs 3 and 5). Shane *et al.* (2003), Li and Liang (2005) and Shen *et al.* (2005) have reported that P concentrations in shoot and phloem sap of white lupin increased at higher P supply, associated with decreased cluster-root formation. Li *et al.* (2008) reported that the formation of cluster roots in *L. albus* is regulated by shoot P concentration, with

a critical level of 2–3 mg g⁻¹. Also, a stepwise reduction of cluster roots at increasing P concentration in the root environment and plant tissues in white lupin has been reported by

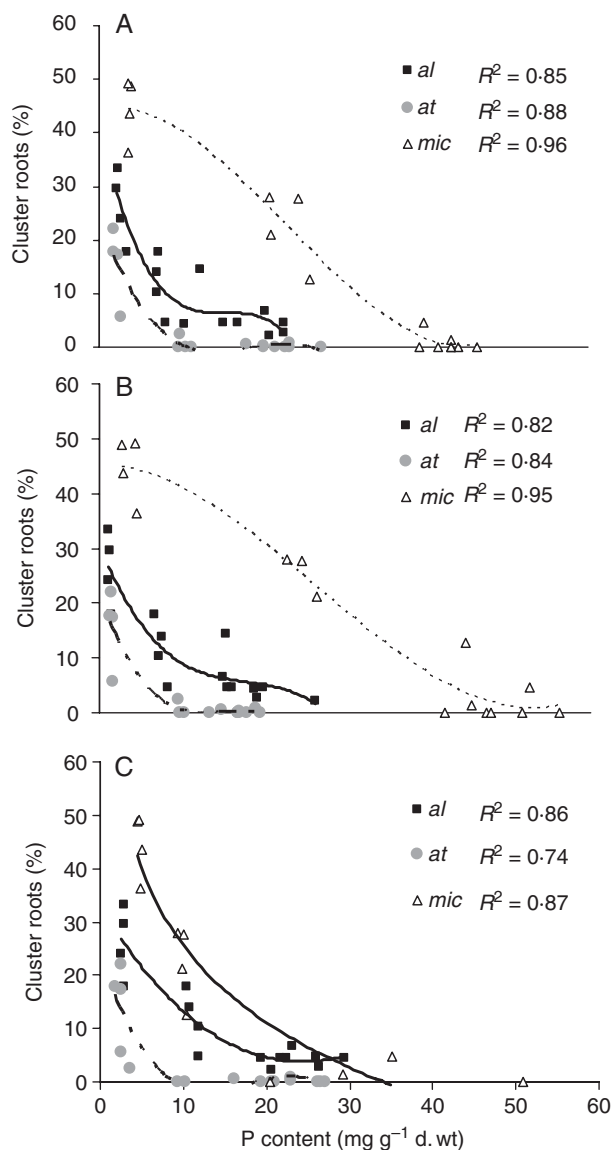


FIG. 5. Relationship between percentage of cluster roots as compared with total roots (based on dry mass) and phosphate concentration in leaves (A), stems (B) and roots (C) of *Lupinus* species. *L. albus* = *al*, *L. atlanticus* = *at* and *L. micranthus* = *mic*.

Keerthisinghe *et al.* (1998). The result for this species, although not as strong as in *L. atlanticus*, confirmed the model by Pearse *et al.* (2006).

In *L. micranthus*, with the lowest relative growth rate, the highest P-uptake rate and, consequently, very high accumulation of P in plant tissues (about 20 mg g⁻¹ d. wt at 10 μM P) was observed (Tables 1 and 2, Fig. 4). A concentration of 1 μM P in the nutrient solution did not induce P deficiency symptoms, suggesting this species is adapted to an environment where P supply is always limiting. However, the reduction in percentage of cluster-root dry mass at 1–10 μM P at the final harvest was 68 % (Table 2, Fig. 3). Also, at least part of this reduction in cluster roots at 10 μM P was related to P toxicity, due to greater sensitivity to P toxicity in this species, suggesting poorer regulation of P uptake. Sensitivity to shoot P status in *L. micranthus* is even less than that of *L. albus*; hence *L. micranthus* responded even less to increasing P supply with respect to reducing allocation to cluster roots. Slow growth and thus little dilution of the internal P concentration may be one explanation for this phenomenon (Lambers and Poorter, 1992). On the other hand, the formation of cluster roots suggests that *L. micranthus* is able to mobilize sparingly soluble P forms in soils. However, in a hydroponic culture system, where P supply is not limited by diffusion or solubility, the formation of cluster roots has limited advantage and P acquisition may rather depend on the properties of P-uptake systems than on root morphological characteristics. Consequently, a threshold P concentration in leaves for partial inactivation of cluster-root formation could not be identified.

A complex series of signalling cascades is emerging that control transcriptional cascades and initiate plant responses to P starvation. Several investigators have reported that P may act directly as a signal and regulate some aspects of plant P-starvation responses, such as root-meristem activity, root-hair development and cluster-root formation (López-Bucio *et al.*, 2003; Zhang *et al.*, 2003; Pearse *et al.*, 2006). Also, it is well known that the balance of plant growth regulators plays a role in the final stage(s) in the signal-transduction pathway (Gilbert *et al.*, 2000). The fine balance between auxin, ethylene and local cytokinin concentrations, their transport from the shoot to the root or changes in the sensitivity of target tissues to these plant regulators may be involved in the control of systemic responses to P starvation (Vance *et al.*, 2003; Hammond and White, 2008). In addition, many recent studies have implicated the involvement of shoot-derived carbohydrate signals (sucrose, glucose and fructose) in

TABLE 2. Comparison of three *Lupinus* species based on growth rate, P uptake and P accumulation, and initiation or suppression of cluster-root formation

	<i>L. micranthus</i>	<i>L. albus</i>	<i>L. atlanticus</i>
Growth rate	Low	Medium	High
Optimum phosphate concentration for the highest growth (μM)	1	10	10–50
Induction of cluster root at 1 μM P supply	High	High	High
Inhibition of cluster roots by higher phosphate supply	Stepwise	Stepwise	Sharply at 10 μmol
Foliar phosphate concentration below 10 μM P in solution (mg g ⁻¹ d. wt)	20–22	4–7	7–10
P uptake rate at 10 μM P in solution (mg P g ⁻¹ d. wt d ⁻¹)	4.81	0.36	2.43
P uptake rate at 1 μM P in solution (mg P g ⁻¹ d. wt d ⁻¹)	0.09	0.06	0.09
Reduction in cluster-root formation at 10 μM P compared with 1 μM P (%)	68	70	98

control of plant P-starvation responses (Liu *et al.*, 2005; Müller *et al.*, 2007; Zhou *et al.*, 2008). A role of the microRNA family as an intermediate component of this signalling cascade regulating P-starvation-induced genes has recently been identified. Members of this family are specifically and rapidly up-regulated by P starvation, but are not detectable under P-replete conditions (Fujii *et al.*, 2005; Bari *et al.*, 2006). Present knowledge suggests that achieving an internal threshold P concentration (Li *et al.*, 2008) is a first step of signalling cascades necessary to stimulate other signals and trigger plant responses (Hammond and White, 2008). The absence of a P-induced suppression of cluster-root formation in *L. micranthus* may therefore also reflect a suppression of an existing control mechanism for cluster-root formation due to interference of P toxicity with the factors mentioned above, induced by an artificially high P uptake under the investigated culture conditions.

Taken together, it is the shoot P status that stimulates downstream signals and regulates cluster-root formation in *L. albus* and *L. atlanticus*, while inhibition of cluster-root production is less responsive to P status in *L. micranthus*. The exact mechanism accounting for this difference needs further elucidation. In addition, the presence of cluster roots does not necessarily reflect a high activity in terms of release of P-mobilizing root exudates; this also warrants further investigation.

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