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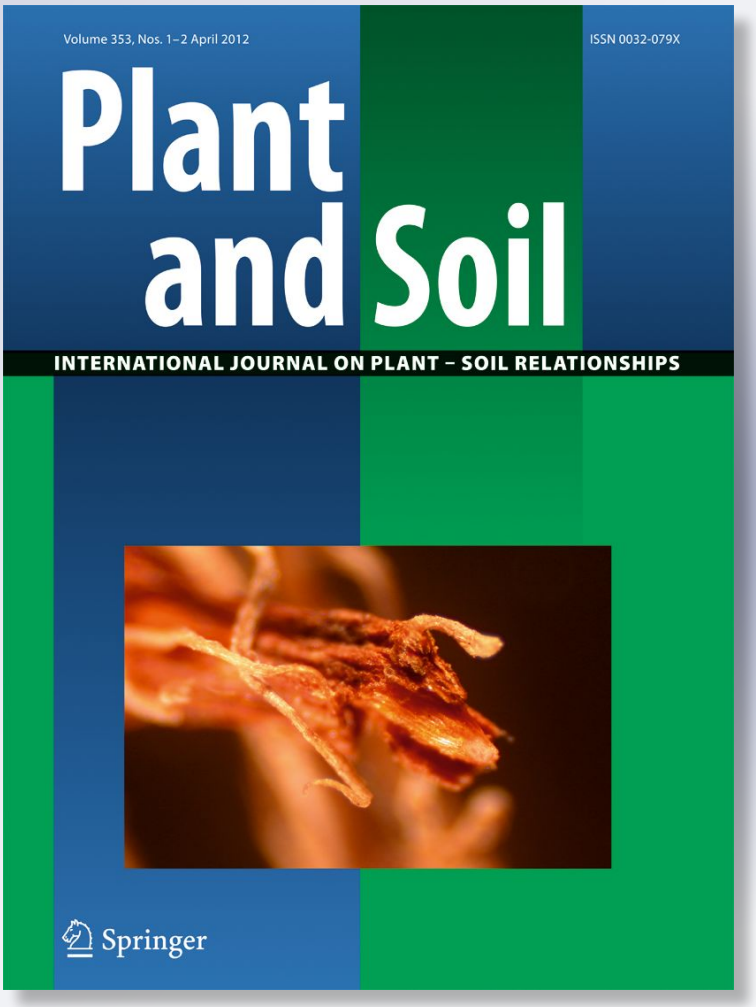

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REGULAR ARTICLE

Assessing variability in root traits of wild *Lupinus angustifolius* germplasm: basis for modelling root system structure

Ying Long Chen · Vanessa M. Dunbabin · Art J. Diggle · Kadambot H. M. Siddique · Zed Rengel

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Abstract
Background and aims Intra-specific variation in root system architecture and consequent efficiency of resource capture by major crops has received recent attention. The aim of this study was to assess variability in a number of root traits among wild genotypes of narrow-leafed lupin (*Lupinus angustifolius* L.), to provide a basis for modelling of root structure.
Methods A subset of 111 genotypes of *L. angustifolius* was selected from a large germplasm pool based on similarity matrices calculated using Diversity Array Technology markers. Plants were grown for 6 weeks in the established semi-hydroponic phenotyping systems to measure the fine-scale features of the root systems.
Results Root morphology of wild *L. angustifolius* was primarily dominated by the taproot and first-order branches, with the presence of densely or sparsely distributed second-order branches in the late growth stage. Large variation in most root traits was identified among the tested genotypes. Total root length, branch length and branch number in the entire root system and in the upper roots were the most varied traits (coefficient of variation CV >0.50). Over 94% of the root system architectural variation determined from the principal components analysis was captured by six components (eigenvalue >1). Five relatively homogeneous groups of genotypes with distinguished patterns of root architecture were separated by *k*-means clustering analysis.
Conclusions Variability in the fine-scale features of root systems such as branching behaviour and taproot growth rates provides a basis for modelling root system structure, which is a promising path for selecting desirable root traits in breeding and domestication of wild and exotic resources of *L. angustifolius* for stressful or poor soil environments.

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Introduction

Narrow-leafed lupin (*Lupinus angustifolius* L.) has the broadest natural distribution of the Mediterranean and North African lupin species, and is an important component of sustainable farming systems in the Mediterranean climatic region, particularly in Australia and some European countries (Gladstones 1974; Clements and Cowling 1994; Palta et al. 2008). In Australia, narrow-leafed lupin is the most important grain legume crop for the stockfeed industry (Dracup and Thomson 2000; Byrne et al. 2010).

Breeding programs have focussed on increasing grain productivity of *L. angustifolius* cultivars, and maintaining low alkaloid and high protein (above 30%) contents in seeds (Buirchell 2008). In Australia, however, large-scale cultivation of narrow-leafed lupin has been restricted in some soil types and rainfall areas. For example, a commercial *L. angustifolius* cultivar 'Gungurru' performed poorly on fine-textured or alkaline soils, probably because of an unsuitable root system characterized by few second-order roots compared with other *Lupinus* species (Clements et al. 1993). The low density of lateral roots may be insufficient for efficient water and nutrient extraction, particularly when the resource distribution and taproot penetration are restricted.

The importance of crop root system architecture (RSA) in capturing resources and the consequent effect on growth and yield has been well-documented (e.g. Lynch 1995; Dunbabin 2007; Gregory et al. 2009; Hammer et al. 2009; Ao et al. 2010). Root architecture plays a vital role in the exploration of soil zones and acquisition of soil nutrients such as P (Lynch and Brown 2001; Rose et al. 2009). To improve adaptation of commercial cultivars of lupins in a wide range of soil types and climatic conditions, new sources of *L. angustifolius* germplasm (including wild types and exotic germplasm from gene banks) can be introduced to investigate intra-specific variations for useful root traits.

A large germplasm pool (1301 genotypes) of *L. angustifolius* has been established in Western Australia, including landraces from diverse locations such as the Mediterranean Basin, Europe and West Asia. Taking advantage of this core collection by selecting germplasm for maximum variability using the DArT method (Diversity Array Technology, which can detect DNA variability in hundreds of loci simultaneously), this research aimed to examine genetic variation in intrinsic root architecture among 111 genotypes. The objectives of this study were therefore to establish an efficient technique to measure the fine-scale features of a root system, determine genetic variation in root architecture, select genotypes with interesting root traits for further examination of resource acquisition, and provide a basis for modelling root system architecture using two prominent root models, ROOTMAP (Diggle 1988; Dunbabin et al. 2002) and SimRoot (Lynch et al. 1997). An economic and efficient growth system as a phenotyping platform was established for this study (Chen et al. 2011a).

Materials and methods

Plant growth and measurement

Three core subsets of wild genotypes of narrow-leafed lupin (*Lupinus angustifolius*) from a large germplasm pool were obtained (i) an Eco subset based on the habitats where these genotypes were collected (similar to the approach used in yellow lupin, *L. luteus* L., Berger et al. 2008); (ii) a DArT subset based on similarity matrices calculated using DArT markers; (iii) an EcoDArT subset is based on the two data sources above and is a compromise between maximising environmental and genetic diversity. One hundred and eleven genotypes from the DArT subset were included in this screening experiment. A list of tested genotypes is available from the authors on request. Local *L. angustifolius* cultivar Marri was used as the control, selected from randomly amplified microsatellite polymorphism (RAMP) analysis (Yuan et al. 2005). Seeds of *L. angustifolius* were scarified by scalpel, sown in pots filled with wet washed river sand and germinated at 22/16°C (day/night) in the dark for 2 day and then in light for another 2 day before transplanting into the phenotyping system.

Plants were cultivated in the semi-hydroponic phenotyping system as described previously (Chen et al. 2011a). Each bin was filled with 30 l of solution

containing (μM): K (1220), P (20), S (1802), Ca (600), Mg (200), Cu (0.2), Zn (0.75), Mn (0.75), B (5), Co (0.2), Na (0.06), Mo (0.03), Fe (20) and N (1000). The plant growth units maintained moisture via an automatic pumping system. Solution was refreshed weekly.

The experiment was undertaken during spring in a temperature-controlled glasshouse in Perth (31°58′S, 115°49′E). The daily average temperature was 22/16°C (day/night), and midday maximum photosynthetic photon flux density was 1,852 μmol photons m^{-2} s^{-1} over the experimental period.

A randomized block design was used consisting of four replicate bin systems each containing three bins and 112 genotypes including the control cultivar 'Marri'. Four replicate plants of each genotype were assigned to four separate bins. Buffer plants were added when required to ensure equal number of plants (40) allocated to each bin. Plant transfer into bins was staggered 1 day apart at establishment for ease of subsequent observation and handling.

Root systems were photographed and lengths of taproots were measured fortnightly. Plants were harvested 6 weeks after transplanting. Shoot height and leaflet number per plant were measured at harvest. Subsamples of roots were collected at harvest by cutting the taproot into 20-cm sections starting from the base for morphological and architectural measurements. Shoots and roots were dried in an air-forced oven at 70°C for 72 h, and weighed to obtain dry mass. Root subsamples were optically scanned before drying (see below).

Image and data analysis

Root subsamples were scanned in greyscale at 300 dpi using a desktop scanner (Epson Expression Scan 1680, Long Beach, Canada). Images were analyzed using WinRHIZO software (v2009, Regent Instruments, Montreal, QC, Canada). The debris removal filter was set to discount objects less than 1 cm^2 with a length/width ratio less than 10. The roots were partitioned into 11 diameter classes: <0.25, 0.25–0.5, 0.5–0.75, 0.75–1.0, 1.0–1.25, 1.25–1.5, 1.5–2.0, 2.0–2.5, 2.5–3.0, 3.0–3.5 and >3.5 mm.

The growth parameters measured included taproot length at 2, 4 and 6 weeks after planting, and leaflet number, shoot height, and root and shoot dry mass at harvest. Root growth rate was based on taproot length increments for each growth period. Data for various root traits, such as total root length, root surface area, root volume, average root diameter and Diameter Class Length (DCL, root length within a diameter class) were generated in WinRHIZO from root images for each root section. Root trait data in the upper 0–20 cm section (referred in this paper as 'top' section) were compared with those for the entire root system. The number of branches (first-order and second-order) in each root section was counted manually. The following parameters were based on observed and/or computed data:

- Root mass ratio (root dry mass/total dry mass)
- Root-to-shoot mass ratio (root dry mass/shoot dry mass)
- Specific root length (SRL) = root length/root dry mass (m g^{-1})
- First-order branch density = number of branches/taproot length (m^{-1})
- Branch intensity = number of branches/root length (m^{-1})
- Root tissue density = root mass/root volume (mg m^{-3})
- Relative Diameter Class Length (rDCL) = DCL/root length (yielding a proportion of root length to normalize disparity between plants of different sizes).

General Linear Model (GLM) multivariate analysis was performed for genotype main effects after non-significant differences between bins and harvesting times were identified in the PASW Statistics 18 (SPSS Inc., Chicago, IL, USA). The multivariate standard error of skewness and kurtosis was 0.23 and 0.45, respectively, when all parameters were included in the GLM analysis, indicating no serious departure from multivariate normality. General correlations between parameters were examined with Pearson correlation coefficients. Correlations were considered statistically significant if $P \leq 0.05$ (*) or $P < 0.01$ (**). Principal component analysis (PCA) was used to identify determinants of root architectural variability across genotypes (Jolliffe 2002). Mean data for seven selected root traits were subjected to *k*-means clustering analysis to generate relatively homogeneous groups of the tested genotypes.

Results

Variation in shoot size and biomass accumulation

Plants of 111 wild genotypes exhibited vigorous growth within the 6-week experimental period when cultivated in our phenotyping system, indicating an appropriate experimental environment for growth of wild *L. angustifolius*. Large variation among genotypes was observed in shoot growth, root proliferation and biomass allocation (Table 1). At harvest, shoot heights ranged from 46 to 244 mm, with an average of 141 mm. Leaflet number varied up to 5 folds between large and small plants. Variation in shoot height and leaflet number resulted in large differences in shoot dry mass among genotypes. Biomass accumulation in root systems and biomass allocation between shoot and root varied among the tested genotypes. The average ratio of root-to-shoot dry mass was 0.6, and root mass over total mass ranged from 0.25 to 0.52. Root dry mass was strongly correlated with shoot dry mass (R^2=0.82, P< 0.01; Fig. 1).

Table 1 Descriptive statistics and analysis of variance for 27 root traits and growth parameters in 111 wild *L. angustifolius* genotypes

Traits	Unit	Minimum	Maximum	Mean	CV	P (n=112)
Root length	cm	99.9	1793.9	415.5	**0.53**	<0.001
Branch length	cm	69.4	1690.2	342.6	**0.62**	<0.001
First-order branch number		16.3	317.0	84.8	0.47	<0.001
Root surface area	cm^2	20.1	586.0	128.1	**0.59**	<0.001
Root diameter	mm	0.61	1.29	0.97	0.16	<0.001
Root volume	cm^3	0.3	16.4	3.3	**0.69**	<0.001
Taproot length	cm	24.5	111.3	70.9	0.23	<0.001
Specific root length	$m\ g^{-1}$	13.9	56.9	23.2	0.35	<0.001
Branch density	m^{-1}	43.7	305.8	120.0	0.38	<0.001
Branch intensity	m^{-1}	7.8	42.8	22.1	0.34	<0.001
Root tissue density	$mg\ cm^{-3}$	34.3	327.6	76.9	0.45	0.139
Branch length/taproot length	$cm\ d^{-1}$	1.1	16.3	4.8	**0.52**	<0.001
Top root length	cm	69.7	889.7	233.6	**0.57**	<0.001
Top branch length	cm	49.7	869.7	213.6	**0.62**	<0.001
Top branch number		14.3	97.3	43.8	0.35	<0.001
Top root length/root length	%	30.9	90.0	56.7	0.20	0.013
Top branch length/branch length	%	33.0	92.7	63.5	0.20	0.012
Top branch number/branch number	%	25.2	87.7	54.6	0.21	0.011
Top branch density	m^{-1}	71.3	486.7	218.9	0.35	<0.001
Root growth rate	$cm\ d^{-1}$	0.58	2.63	1.72	0.23	<0.001
Leaflet number	$plant^{-1}$	70.0	362.5	145.9	0.34	<0.001
Shoot height	mm	46.3	243.8	141.1	0.29	<0.001
Shoot dry mass	$mg\ plant^{-1}$	93.5	1441.8	385.3	**0.56**	<0.001
Root dry mass	$mg\ plant^{-1}$	42.5	1094.3	248.5	**0.61**	<0.001
Total dry mass	$mg\ plant^{-1}$	136.0	2536.0	633.8	**0.57**	<0.001
Root dry mass/shoot dry mass		0.33	1.09	0.65	0.24	0.065
Root dry mass/total dry mass	%	24.5	52.1	38.6	0.15	0.001

Minimum and maximum values, mean and coefficient of variation (CV) are given for each parameter. Traits with CV values ≥0.50 appear in bold type. Probability values (P) were based upon a GLM multivariate analysis of 112 genotypes. Top root length, root length in the upper 20-cm root section; Specific root length, root length per unit mass; Branch density, numbers of branches per taproot length; Branch intensity, number of branches over total root length; Root tissue density, root mass per unit root volume. Root growth rate was calculated based on taproot growth over the 6 weeks observation period

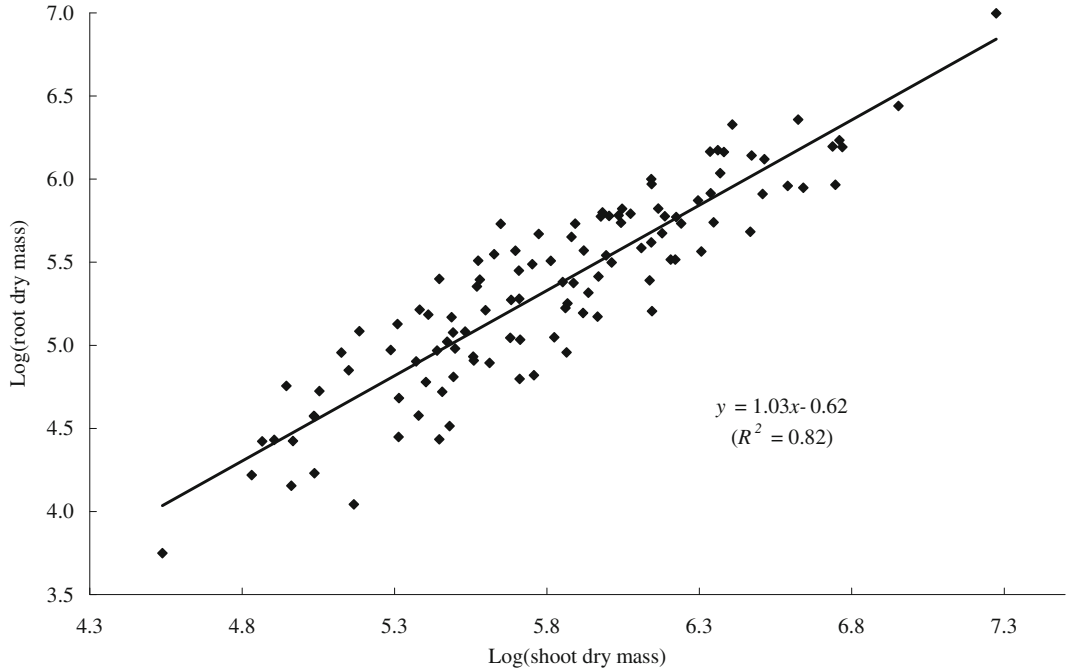

Fig. 1 Correlation between root and shoot dry mass of 111 wild *L. angustifolius* genotypes 6 weeks after planting ($P<0.01$). Data are logarithmic means of four replicates

Rooting pattern

The root system of tested genotypes was dominated by the taproot and primary lateral roots (=first-order branches). Significant differences in the rooting pattern and branching type were observed among genotypes. Root architectural and morphological traits, including root length, branch length, and root area and volume displayed large variation among genotypes, with the coefficient of variation (CV) greater than 0.5 in each trait ($P<0.01$; Table 1). Some genotypes had specific root traits, such as long first-order branches, high branch density in the upper part of the root system (i.e. top section, 0–20 cm) and/or the lower part, long taproot with sparse and short branches, abundant second-order branches, or dense root hairs.

Notable variation in top branch length was detected among genotypes (CV = 0.62, $P<0.01$; Table 1). On average, the top roots had 64% of total branch length ($P=0.01$) and 55% of total branch number ($P=0.01$). All genotypes generally had relatively higher branch density in the top root system (219 branches m^{-1} root) compared to the lower part, resulting in an average of 120 branches m^{-1} root for the entire root system.

Root growth dynamics

The dynamics of root growth varied among genotypes. Root growth rate, based on taproot elongation, ranged from 0.6 to 2.6 cm d^{-1} over the 6-week growth period, with an average of 1.7 cm d^{-1} for all genotypes tested (Table 1). This value is somewhat lower than that for the control cultivar 'Marri' which grew 2.1 cm d^{-1}. Furthermore, relatively wider variation in taproot growth was observed in the later stages. Nevertheless, elongation of taproots in the later growth periods (4- and 6-week after planting) was correlated with that measured in the first 2 weeks ($P<0.01$; data not shown).

Root diameter and diameter class length

A low coefficient of variation was identified for root diameter among genotypes (CV = 0.16, $P<0.01$; Table 1). The data for relative diameter class length (rDCL) had approximately 50% of total root length in the diameter classes between 0.5 and 1.0 mm (Fig. 2), of which 29% was in the 0.50–0.75 mm class. The 0.25–0.5 mm diameter class contained 14% of total root length. Roots thicker than 2.0 mm were mostly

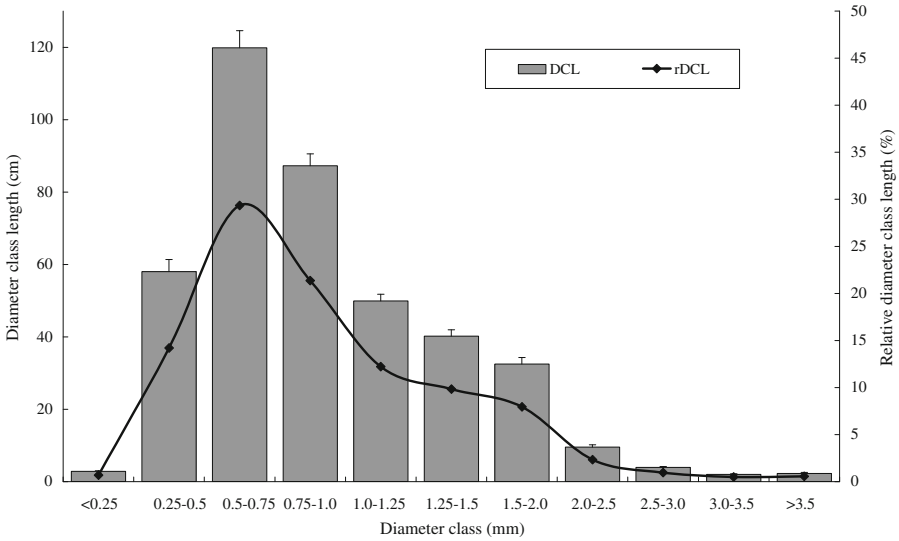

Fig. 2 Root diameter class length (DCL, mean + SE) (*bars*) and relative diameter class length (rDCL) (*line*) of 111 wild *L. angustifolius* genotypes 6 weeks after planting

proximal (the top root part near shoot), accounting for approximately 4% of total root length.

General correlations among root traits

Pearson correlation matrix was established to identify correlation among root traits. A subset of 14 root architectural and morphological traits and four growth parameters were selected with relatively large coefficients of variation (Table 1). There was strong correlation among most selected traits (Table 2). Total root length was strongly correlated with each of the 13 other root traits at $P<0.01$ (0.03 for specific root length). Root length increased as branch length, branch number, root area, volume, branch density and branch length/taproot length ratio increased ($P<0.01$; Table 2). Root length was also highly correlated with the major traits of the top roots, including top root length and branch number ($P<0.01$). Branch length displayed similar correlations as root length (Table 2). Top branch length was the same as top root length in terms of correlation with other traits, and top branch density followed the top branch number. High values of specific root length positively correlated with large branch intensity ($P<0.01$, respectively), small root area or volume, but was not significantly associated with other root traits (Table 2).

Allometry between root traits and growth parameters

Correlation between root traits and growth parameters varied for each pair (Table 2). Root length significantly correlated with leaflet number, root mass, shoot mass and total biomass (all $P<0.01$). Figure 3 illustrates strong correlation between root length and biomass accumulation ($R^2=0.74$, $P<0.01$). Similarly, other root traits such as branch length and number, and root area and volume were highly correlated with each of the four selected growth parameters. Taproot length measured at harvest was correlated with growth parameters ($P<0.01$; data not shown). Root length showed low correlation with taproot growth rate ($R^2=0.21$, $P<0.01$; Fig. 4).

Determination of trait variation with principal components

Eighteen root traits were included in the principal component analysis, resulting in six principal components (PC) with eigenvalues greater than 1 (Table 3) that captured more than 94% of the root system architecture variation across 111 *L. angustifolius* genotypes. The first component (PC1) represented 36% of the variability, and consisted mostly of the whole-root-system traits, including root length and branch length in the entire root system or the top roots, total surface area and root volume, ratio of branch length and taproot length, and branch intensity (Table 3). PC2 (19% variation) accounted primarily for branch number and branch density. Thirteen percent of the variation, accounted for by PC3, was mainly derived from taproot length and its associated root growth rate. PC4 accounted for 12% of the variation, mostly from ratios of top branch length and top branch

Table 2 Pearson's correlation matrix for 18 traits characterizing root architecture, morphology and biomass accumulation in 111 wild *L. angustifolius* genotypes

	Root length	Branch length	Branch number	Root area	Root volume	Specific root length	Branch density	Branch intensity	Root tissue density	Branch length/tap-root length	Top root length	Top branch length	Top branch number	Top branch density	Leaflet number	Shoot dry mass	Root dry mass	Total dry mass
Root length		0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Branch length	**1.00**		0.00	0.00	0.00	0.04	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Branch number	**0.76**	**0.75**		0.00	0.00	0.36	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Root area	**0.97**	**0.97**	**0.68**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Root volume	**0.91**	**0.90**	**0.60**	**0.98**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Specific root length	*−0.18*	*−0.17*	−0.03	**−0.29**	**−0.34**		0.15	0.00	0.39	0.16	0.03	0.03	0.14	0.14	0.05	0.01	0.00	0.00
Branch density	**0.57**	**0.59**	**0.83**	**0.47**	**0.37**	0.10		0.00	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Branch intensity	**−0.41**	**−0.42**	*0.22*	**−0.45**	**−0.47**	**0.27**	**0.27**		0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Root tissue density	**−0.22**	*−0.20*	*−0.21*	**−0.28**	**−0.31**	−0.03	−0.06	0.06		0.12	0.06	0.06	0.04	0.04	0.06	0.02	0.03	0.02
Branch length/tap-root length	**0.87**	**0.90**	**0.57**	**0.82**	**0.74**	−0.10	**0.66**	**−0.48**	−0.11		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Top root length	**0.91**	**0.92**	**0.52**	**0.91**	**0.86**	*−0.18*	**0.41**	**−0.55**	−0.15	**0.86**		0.00	0.00	0.00	0.00	0.00	0.00	0.00
Top branch number	**0.91**	**0.92**	**0.52**	**0.91**	**0.86**	*−0.18*	**0.41**	**−0.55**	−0.15	**0.86**	**1.00**		0.00	0.00	0.00	0.00	0.00	0.00
Top branch length	**0.56**	**0.55**	**0.84**	**0.47**	**0.38**	0.10	**0.86**	**0.32**	*−0.17*	**0.51**	**0.40**	**0.40**		0.00	0.00	0.00	0.00	0.00
Top branch density	**0.56**	**0.55**	**0.84**	**0.47**	**0.38**	0.10	**0.86**	**0.32**	*−0.17*	**0.51**	**0.40**	**0.40**	**1.00**		0.00	0.00	0.00	0.00
Leaflet number	**0.75**	**0.75**	**0.59**	**0.71**	**0.64**	*−0.16*	**0.46**	**−0.31**	−0.15	**0.67**	**0.65**	**0.65**	**0.48**	**0.48**		0.00	0.00	0.00
Shoot dry mass	**0.83**	**0.83**	**0.66**	**0.82**	**0.77**	**−0.23**	**0.53**	**−0.27**	*−0.19*	**0.73**	**0.74**	**0.74**	**0.49**	**0.49**	**0.85**		0.00	0.00
Root dry mass	**0.86**	**0.86**	**0.60**	**0.89**	**0.88**	**−0.37**	**0.43**	**−0.38**	*−0.18*	**0.73**	**0.80**	**0.80**	**0.38**	**0.38**	**0.79**	**0.92**		0.00
Total dry mass	**0.86**	**0.86**	**0.64**	**0.87**	**0.83**	**−0.30**	**0.50**	**−0.32**	*−0.19*	**0.75**	**0.78**	**0.78**	**0.45**	**0.45**	**0.84**	**0.99**	**0.97**	

Correlation is significant at the 0.01 level (bold) or 0.05 level (italic). Proportion values are given on top right side of matrix table. Traits with CV value ≥0.30 were included in the analysis (see Table 1 for CV values)

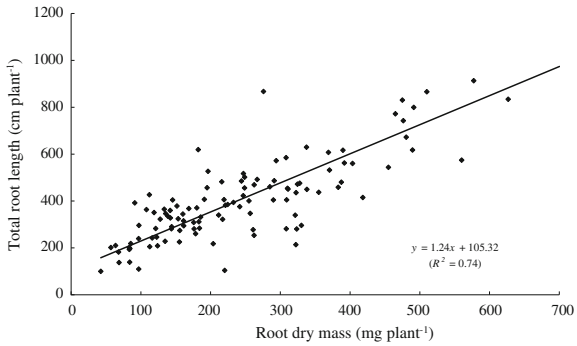

Fig. 3 Relationship between root biomass accumulation and total root length in 111 wild *L. angustifolius* genotypes. Mean values of four replicates are presented

number to that in the whole root system. The first four PCs comprised 80% of the total variability. Specific root length and diameter are the major contributors to PC5 with 8% of the variation. PC6 accounted for 7% of root system architectural variability, coming mostly from root tissue density.

Plots of genotype distribution for some selected combinations of the six PCs are presented in Fig. 5. Relative distance between the tested genotypes was displayed for each combination of root traits. In loading plots of PC1 vs. PC2 to PC6 (Fig. 5a–e, respectively), one genotype (i.e. DArT#85) appeared as an outlier having the largest root system in terms of root length and branch length. PC1 vs. PC2 (Fig. 5a) explained up to 55% of total variance. Figure 5a illustrates how the genotypes vary in root length and branch length (separated by PC1), and branch number and branch density (by PC2). The 2-D presentation of PC3 against PC1 (Fig. 5b) accounts for 49% of RSA variability, including 13% genotypic variation in taproot length (PC3). The plot shows that genotypes with large root systems do not necessarily produce deep taproots, and vice versa. PC4 in Fig. 5c demonstrates variation in the properties of the upper roots. The plot of PC1 vs. PC5 (Fig. 5d) separates genotypes into different specific root lengths and diameters in addition to the size of the root system, accounting for 43% of total variance. Figure 5e presents variation in root tissue density (PC6), suggesting generally low variance among genotypes. The loading plots of PC2 vs. PC3 (Fig. 5f) illustrate large variation among genotypes with respect to branch density (PC2) and taproot length (PC3), with these two components accounting for 32% of total variance.

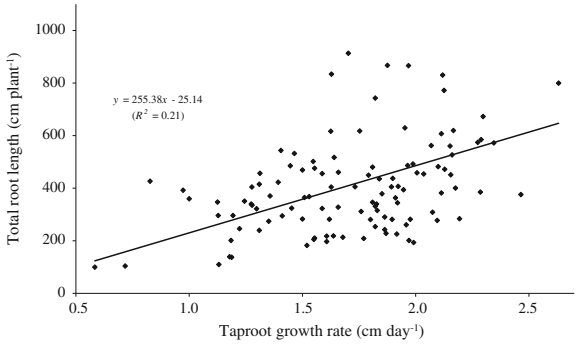

Fig. 4 Relationship between root length and taproot growth rate in *L. angustifolius*. Values presented are means of four replicates

Identification of groups of genotypes with relatively homogeneous root traits

Five relatively homogeneous groups of genotypes were determined by *k*-means clustering analysis involving the seven most important root traits (Table 4). Six traits contributed significantly to group separation ($P<0.01$), whilst specific root length contributed the least ($P=0.13$). Root length had the greatest separation between clusters with the largest F value. Top root length also contributed significantly, along with branch density and branch number. The number of genotypes in each of the five clusters varied from one to 44, indicating the variation in a degree of homogeneity among tested genotypes (Table 4). The outlier genotype, DArT#085, having the largest root system with about 18 m of total root length and more than 300 first-order branches, was separated from the others (Cluster A). Cluster B had eight genotypes with the second largest root system in terms of root length, branch number and branch density. The analysis grouped 35 genotypes (32%, Cluster E) with the smallest root systems and the lowest cluster means in all traits, except for specific root length. The other two groups, consisting of 60% of all genotypes with moderately-sized root systems characterized by similar root diameter, taproot length and specific root length, were positioned between Clusters B (greater root length and branch density) and E. The cluster analysis confirmed approximately 37% of the tested wild genotypes had root systems larger than the control cultivar ‘Marri’. Wild

Table 3 Variable loading scores of 18 root traits and proportion of variation of each principal component

	PC1	PC2	PC3	PC4	PC5	PC6
Top root length	**0.94**	0.16	0.15	0.22	0.04	0.02
Branch length	**0.92**	0.30	0.18	−0.11	0.04	0.03
Branch length/taproot length	**0.92**	0.27	−0.23	−0.06	0.00	0.01
Root length	**0.91**	0.30	0.24	−0.12	0.05	0.04
Root area	**0.90**	0.22	0.27	−0.07	0.18	0.13
Root volume	**0.85**	0.15	0.28	−0.03	0.27	0.20
Branch intensity	**−0.66**	0.57	0.10	−0.19	−0.17	−0.13
Top branch number	0.28	**0.94**	0.07	−0.10	−0.05	0.08
Branch density	0.41	**0.80**	−0.20	−0.30	−0.10	−0.09
Branch number	0.47	**0.73**	0.31	−0.34	−0.03	−0.03
Taproot length	0.16	0.09	**0.92**	−0.19	0.14	0.17
Root growth rate	0.21	0.05	**0.90**	−0.18	0.12	0.18
Top branch length/branch length	0.08	−0.31	−0.11	**0.91**	−0.04	−0.12
Top root length/root length	0.25	−0.25	−0.28	**0.85**	−0.04	−0.15
Top branch number/branch number	−0.37	0.02	−0.55	**0.60**	−0.07	0.10
Specific root length	−0.15	0.11	−0.11	0.06	**−0.93**	0.12
Root diameter	0.22	−0.12	0.28	−0.05	**0.67**	0.55
Root tissue density	−0.12	−0.06	−0.19	0.15	0.04	**−0.91**
Eigenvalue	7.2	3.8	2.7	2.4	1.5	1.3
Variability (%)	35.8	19.0	13.3	12.0	7.5	6.7
Cumulative variability (%)	35.8	54.8	68.1	80.1	87.7	94.4

Rotation converged in 7 iterations using Varimax with Kaiser Normalization method. Variable loading scores ≥0.50 for each component appear in bold. Principal components with eigenvalues >1 are presented and considered significant (Tabachnik and Fidell 1996)

genotypes generally had thinner roots with more specific root length than the local cultivar (Table 4).

Discussion

Wild *L. angustifolius* genotypes differed in root size and growth rate

The root systems of wild *L. angustifolius* genotypes primarily consisted of taproot and first-order lateral roots, with the presence of densely or sparsely distributed second-order branches appearing in the final measurement period. This result is consistent with our recent observations for wild *L. angustifolius* established in the same growth system (Chen et al. 2011a, b). The tap-root dominant rooting pattern of *L. angustifolius* was also found for the commercial cultivar 'Gungurru' grown in river sand (Clements et al. 1993) and in nutrient solution (Dunbabin et al. 2002). The rooting pattern of *L. angustifolius* differs significantly from other lupin species including *L. albus*, *L. atlanticus*, *L. leuteus*, *L. micranthus*, *L. mutabilis*, *L. palaestinus* and *L. pilosus*, where dominant tertiary lateral roots with a shorter and thinner taproot are produced (Bishop et al. 1986; Clements et al. 1993).

This investigation showed large genotypic variation in root growth rate, proliferation and branching among wild genotypes. The tested wild genotypes had generally lower root growth rate and smaller total root length when compared with the control cultivar 'Marri'. Genotypic variation in root length was reported for 30 wild accessions of *L. angustifolius* in solution culture, root length ranged from 1.2 to 6.1 m (pH 5.2) and 0.7 to 4.0 m (pH 7.0) (Tang and Robson 1998). The total root length of wild genotypes in neutral pH (2.5 m on average) was smaller than that of the two Australian cultivars (Marri and Yandee, 2.8 m), whilst larger (3.3 m) in pH 5.2 than the cultivars (2.7 m). Römer et al. (2000) reported longer roots (i.e. total root length) for two German cultivars 'Bordako' and 'Borweta' compared to the wild genotypes. As far as we know, there is no other available data on root length for wild genotypes of *L. angustifolius* grown in any conditions. However, it is

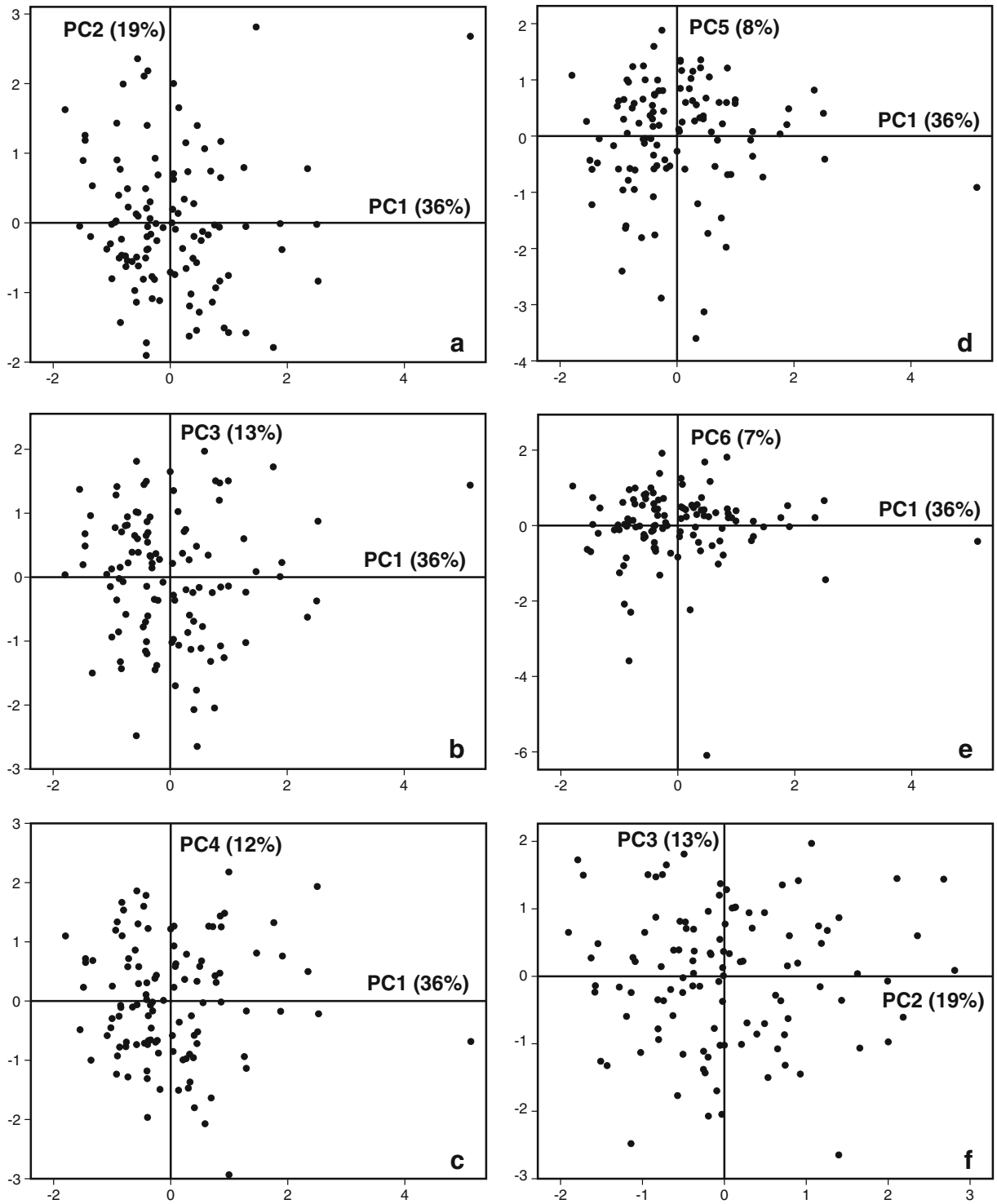

Fig. 5 Principal component analysis of root system architecture variation in *L. angustifolius*. Eighteen root traits were used to analyze the variation across 111 wild genotypes (see Table 3). The position of each genotype in 2-D plots (i.e. DArT number) is shown for principal component PC2 vs. PC1, representing 55% of the variability (**a**); PC3 vs. PC1 (39%, **b**); PC4 vs. PC1 (48%, **c**); PC5 vs. PC1 (43%, **d**); PC6 vs. PC1 (43%, **e**); and PC3 vs. PC2 (32%, **f**)

Table 4 Cluster centres of five groups generated by *k*-means clustering analysis for seven root traits in 111 wild *L. angustifolius* genotypes. Mean data for 'Marri' (control cultivar) included for comparison

Root traits	Cluster A	Cluster B	Cluster C	Cluster D	Cluster E	Marri	*F*	*P*
Root length	**1794**	812	549	383	*228*	562	327	<0.001
Branch number	**317**	116	107	82	*59*	122	31	<0.001
Diameter	0.99	1.04	1.04	0.97	*0.90*	**1.22**	3.6	0.008
Taproot length	**104**	79	77	69	*65*	87	3.9	0.005
Specific root length	25	19	21	24	**26**	*14*	1.8	0.125
Branch density	**303**	159	147	124	*96*	137	13	<0.001
Top root length	**890**	527	304	211	*126*	339	161	<0.001
Members (%)	1 (0.9%)	8 (7.2%)	23 (20.7%)	44 (39.6%)	35 (31.5%)			

Analysis of variance *F* statistics was performed, and both *F* and *P* values for each variable are given. Traits with large *F* values provide the greatest separation among clusters. For each trait, minimums are italicized and maximums are in bold type

expected that wild genotypes may produce root systems more diverse in size than domesticated cultivars. The large genotypic variation in the root systems documented here demonstrates the potential for identifying suitable/desirable root traits for a range of growth environments in the future.

Wild *L. angustifolius* genotypes displayed complex correlations among root traits

Root length, or a root length profile with depth, is the root characteristics most commonly measured in cropping experiments (e.g. Merrill et al. 2002; Manschadi et al. 2008). This study demonstrated a great deal of variability not just in root length but in other fine-scale root features. The phenotyping system we developed (Chen et al. 2011a) allowed these root properties to be measured in a relatively cost-effective and rapid manner. In addition to root length (used in many studies to reflect the size of the root system), a wide range of root traits including branch length, specific root length and branch density were measured in this investigation, and substantial genotypic variation was found. Together with root morphological and architectural characters, root biomass and associated parameters such as root tissue density (root mass per unit root volume) and specific root length (root length per unit mass) are also important in studying root system architecture. Root tissue density might affect root morphology and root longevity (Eissenstat et al. 2000), and specific root length could characterize economic aspects of the root system and indicate environmental changes (Ostonen et al. 2007). Root hair density is also a significant trait with respect to increased absorption area of a root system (Nielsen et al. 2001). In the present study, numerous root hairs were observed in some genotypes, mostly on second-order branches. Using visual evaluation methods (cf. Zhu et al. 2010), we found that root hair length and density varied largely among selected wild genotypes of *L. Angustifolius,* which may indicate different strategies related to water and nutrient acquisition (Chen et al., unpublished data).

It is generally accepted that root systems are complex (Lynch 1995; Hodge 2010). Understanding the comprehensive multiple relationships among root traits may help to characterize those traits suitable for targeted genotype selection and breeding. Principal component analysis (i.e. Fig. 5) revealed the relative contribution of individual traits to genotypic variation, and highlighted genotype groups that could be crossed to identify the genetic basis of specific root traits.

Together with other experiments (Chen et al. 2011a, b), we established an efficient method to determine fine-scale features of the root system, such as branching behaviour and taproot growth rates that are under direct genetic control and hence have the potential for use in breeding efforts to produce superior root properties (Casson and Lindsey 2003; de Dorlodot et al. 2007). Analysis of quantitative trait loci (QTL) enables identification of specific regions on the genome that are responsible for variation in particular quantitative traits (Weih et al. 2006). Marker-assisted selection and QTL cloning for the root system architecture in wild *L. angustifolius* are in

progress, exploiting genomic resources, candidate genes and the knowledge gained from *Arabidopsis* (Sergeeva et al. 2006), rice (*Oryza sativa* L.) (Horii et al. 2005; Steele et al. 2007) and maize (*Zea mays* L.) (Giuliani et al. 2005). Comprehensive identification of microsatellite markers of the wild genotypes of *L. angustifolius* has been achieved (e.g. Nelson et al. 2010). Combining phenotypic data from the root system architecture and QTLs will enable us to examine the inheritance of root traits.

Wild *L. angustifolius* genotypes had varied traits governing root system architecture

It is anticipated that root morphological and physiological responses largely depend on the scale of heterogeneity (Hodge et al. 2009; Hodge 2010). In the present study, principal component analysis on a subset of root traits captured 94% of RSA variation across all tested genotypes in the six components (eigenvalues >1; Table 3). This method was used to investigate root trait variation among 25 co-existing North American forest species, suggesting the significance of principal components in root studies (Comas and Eissenstat 2009). Variations in root system architecture have been found among crop species and among genotypes of the same species, which may result in differences in root functioning (Rengel and Damon 2008). Genotypic differences in rooting patterns have a major impact on the efficient acquisition of water and nutrients from soils (e.g. Nibau et al. 2008; Rengel and Damon 2008; Rose et al. 2009). For example, root density in the upper soil layers is the most important trait associated with improved acquisition of relatively immobile nutrients such as phosphorus (Manske et al. 2000). Plant root systems are astonishingly plastic in their architecture, which allows for optimal exploitation of diverse soil structures and conditions. Therefore, some caution should be exercised in translating results of root length screens from non-solid media to what might happen in field conditions, and in verifying the screening system adopted (Gregory et al. 2009). Considerably consistent rankings of 10 selected genotypes between this study and two follow-up experiments using either the same semi-hydroponic phenotyping system (Chen et al. 2011a), or soil media, confirmed the capacity of the semi-hydroponic system to represent growth conditions in simple, uniform soil environments (Chen et al. 2011b). The consequent experiments demonstrated that root traits such as root length and branch density are primarily genetically determined, whilst phenotypic plasticity in root–shoot biomass allocation, inflorescence length and leaflet number was found between growth media (Chen et al. 2011b).

Genotypic variation in root traits provides basis for modelling root system structure

Root architecture variation is likely to be associated with genotypic adaptation to contrasting environments. Environment changes may mediate adaptation of plants to soils in which nutrient availability is dependent on alteration of root system architecture, such as increasing root absorption area (López-Bucio et al. 2003). Manschadi et al. (2008) claimed that wheat (*Triticum aestivum* L.) cultivars grown in the Mediterranean environments of southern and western Australia tended to have a wide root angle between the first pair of seminal roots, whereas drought-tolerant genotypes with higher yields in the northern cropping region exhibited narrower angular spread of seminal axes. Genotypes with large, shallow root systems and with greater potential for water extraction early in the season in order to reduce unproductive soil evaporation, are suggested to be more suitable for Mediterranean environments.

Desirable phenotypic traits to increase efficiency of resource capture from drying soil environments remain unknown (Walk et al. 2006). However, defining optimal root systems is problematic, as illustrated by our recent work with root system architectural models: ROOTMAP (e.g. Diggle 1988; Dunbabin et al. 2002; Dunbabin 2007) and SimRoot (e.g. Lynch et al. 1997; Nielsen et al. 1997; Lynch and Brown 2001).

Using a split-root nutrient solution method, Dunbabin et al. (2001) claimed that *L. angustifolius* and *L. pilosus* representing the two extremes of root morphology types present across lupin germplasm (Clements et al. 1993) responded differently to heterogeneous nitrate supply. The result indicates the potential for developing a genotype capable of greater nitrate capture from the soil profile. A model of three-dimensional root growth has been developed to simulate interactions between root systems, and water and nitrate in the rooting environment by using an

external supply/internal-demand regulation system for the allocation of endogenous plant resources (Dunbabin et al. 2002), involving a number of process modules such as ROOTMAP for root growth and structure (Diggle 1988), APSIM's water and nitrogen modules (Probert et al. 1998) and solute module (Rose et al. 1982). Modelling suggested that to improve the ability of *L. angustifolius* to capture nitrate, the density of the first-order laterals should be increased, and there should be a more rapid development of root length density in the topsoil in the first 2 months of growth (Dunbabin et al. 2003).

Data on the various root traits collected in the present investigation have been used to simulate three-dimensional root architecture using ROOTMAP and SimRoot models. Modelling exercises have been made in particular for the 10 selected wild genotypes (DArT#004, 016, 024, 044, 060, 069, 071, 084, 085 and 120), where interesting root traits were identified (Chen et al. 2011b). Furthermore, interactions between root systems and their spatially and temporally heterogeneous environment can be simulated to assess root growth response to water and nutrient supply. Glasshouse experiments aiming to examine the response of rhizosphere exudation of varied root systems of selected wild *L. angustifolius* to phosphorus applications are under investigation alongside modelling exercises.

Conclusions

This study identified wide variation in root system architecture across a substantial subsample of the wild *L. angustifolius* germplasm. For the first time, wild *L. angustifolius* genotypes with vastly different root systems were characterized for further studies ultimately aimed at breeding lines with root traits for improved adaptation to specific environments. This study and follow-up investigations through field or glasshouse trials using root models and QTL analysis are expected to identify candidate genotypes with suitable root traits from wild and exotic resources of *L. angustifolius* for potential breeding for efficient water and nutrient capture in stressful or poor soil environments.

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References

Ao J, Fu AJ, Tian J, Yan X, Liao H (2010) Genetic variability for root morph-architecture traits and root growth dynamics as related to phosphorus efficiency in soybean. Funct Plant Biol 37:304–312

Berger JD, Adhikari KN, Wilkinson D, Buirchell BJ, Sweetingham MW (2008) Ecogeography of the Old World lupins 1 ecotypic variation in yellow lupin (*Lupinus luteus* L). Aust J Agric Res 59:691–701

Bishop A, Delane R, Hamblin J (1986) Root characteristics in branching and reduced branching lupins. In: Proceedings of 4th International Lupin Conference, 15–22 August, 1986. Geraldton, Western Australian. International Lupin Association, Canterbury, New Zealand, pp. 313

Buirchell BJ (2008) Narrow-leafed lupin breeding in Australia–Where to from here? In: Palta JA, Berger JB (eds) Lupins for Health and Wealth–Proceedings of the 12th International Lupin Conference. 14–18 Sept. 2008, Fremantle, Western Australia. International Lupin Association, Canterbury, pp 226–230

Byrne F, Robertson MJ, Bathgate A, Hoque Z (2010) Factors influencing potential scale of adoption of a perennial pasture in a mixed crop-livestock farming system. Agric Syst 103:453–462

Casson SA, Lindsey K (2003) Genes and signalling in root development. New Phytol 158:11–38

Chen YL, Dunbabin VM, Diggle AJ, Siddique KHM, Rengel Z (2011a) Development of a novel semi-hydroponic phenotyping system for studying root architecture. Funct Plant Biol 38:355–363

Chen YL, Dunbabin VM, Postma J, Diggle AJ, Lynch JP, Siddique KHM, Rengel Z (2011b) Phenotypic variation and modelling of wild *Lupinus angustifolius* germplasm. Plant Soil 348:345–364. doi:10.1007/s11104-011-0939-z

Clements JC, Cowling WA (1994) Patterns of morphological diversity in relation to geographical origins of wild *Lupinus angustifolius* from the Aegean region. Gen Resour Crop Evol 41:109–122

Clements JC, White PF, Buirchell BJ (1993) The root morphology of *Lupinus angustifolius* in relation to other *Lupinus* species. Aust J Agric Res 44:1367–1375

Comas LH, Eissenstat DE (2009) Patterns in root trait variation among 25 co-existing North American forest species. New Phytol 182:919–928

de Dorlodot S, Forster B, Pagès L, Price A, Tuberosa R, Draye X (2007) Root system architecture, opportunities and constraints for genetic improvement of crops. Trends Plant Sci 12:474–481

Diggle AJ (1988) ROOTMAP—a model in three-dimensional co-ordinates of the growth and structure of fibrous root systems. Plant Soil 105:169–178

Dracup M, Thomson B (2000) Narrow-leafed lupins with restricted branching. Ann Bot 85:29–35

Dunbabin VM (2007) Simulating the role of rooting traits in crop–weed competition. Field Crop Res 104:44–51

Dunbabin VM, Rengel Z, Diggle A (2001) *Lupinus angustifolius* has a plastic uptake response to heterogeneously supplied nitrate while *Lupinus pilosus* does not. Aust J Agric Res 52:505–512

Dunbabin VM, Diggle AJ, Rengel Z, van Hugten R (2002) Modelling the interactions between water and nutrient uptake and root growth. Plant Soil 239:19–38

Dunbabin VM, Diggle AJ, Rengel Z (2003) Is there an optimal root architecture for nitrate capture in leaching environments? Plant Cell Environ 26:835–844

Eissenstat DM, Wells CE, Yanai RD, Whitbeck JL (2000) Building roots in a changing environment, implications for root longevity. New Phytol 147:33–42

Giuliani S, Sanguineti MC, Tuberosa R, Bellotti M, Salvi S, Laudi P (2005) Root-ABA1, a major constitutive QTL, affects maize root architecture and leaf ABA concentration at different water regimes. J Exp Bot 56:3061–3070

Gladstones JS (1974) Lupins of the Mediterranean region and Africa. West Aust Depart Agr Tech Bull 26:1–48

Gregory PJ, Bengough AG, Grinev D, Schmidt S, Thomas WTB, Wojciechowski T, Young IM (2009) Root phenomics of crops, opportunities and challenges. Funct Plant Biol 36:922–929

Hammer GL, Dong Z, McLean G, Doherty A, Messina C, Schussler J, Zinselmeier C, Paszkiewicz S, Cooper M (2009) Can changes in canopy and/or root system architecture explain historical maize yield trends in the US corn belt? Crop Sci 49:299–312

Hodge A (2010) Roots, the acquisition of water and nutrients from the heterogeneous soil environment. Prog Bot 71: 307–337

Hodge A, Berta G, Doussan C, Merchan F, Crespi M (2009) Plant root growth, architecture and function. Plant Soil 321:153–187

Horii H, Nemoto K, Miyamoto N, Harada J (2005) Quantitative trait loci for adventitious and lateral roots in rice. Plant Breed 125:198–200

Jolliffe IT (2002) Principal component analysis, 2nd edn. Springer, New York

López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L (2003) The role of nutrient availability in regulating root architecture. Curr Opin Plant Biol 6:280–287

Lynch J (1995) Root architecture and plant productivity. Plant Physiol 109:7–13

Lynch JP, Brown KM (2001) Topsoil foraging—an architectural adaptation of plants to low phosphorus availability. Plant Soil 237:225–237

Lynch J, Nielsen K, Davis R, Jablokow A (1997) Simroot, modeling and visualization of botanical root systems. Plant Soil 188:139–151

Manschadi AM, Hammer GL, Christopher JT, de Voil P (2008) Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (*Triticum aestivum* L.). Plant Soil 303:115–129

Manske GGB, Ortiz-Monasterio JI, Ginkel MV, González RM, Rajaram S, Molina E, Vlek PLG (2000) Traits associated with improved P-uptake efficiency in CIMMYT's semi-dwarf spring bread wheat grown on an acid Andisol in Mexico. Plant Soil 221:189–204

Merrill SD, Tanaka DL, Hanson JD (2002) Root length growth of eight crop species in Haplustoll soils. Soil Sci Soc Am J 63:913–923

Nelson MN, Moolhuijzen PM, Boersma JG, Chudy M, Lesniewska K, Bellgard M, Oliver RP, Swiecicki W, Wolko B, Cowling WA, Ellwood SR (2010) Aligning a new reference genetic map of *Lupinus angustifolius* with the genome sequence of the model legume, *Lotus japonicus*. DNA Res 17:73–83

Nibau C, Gibbs DJ, Coates JC (2008) Branching out in new directions, the control of root architecture by lateral root formation. New Phytol 179:595–614

Nielsen KL, Lynch JP, Weiss HN (1997) Fractal geometry of bean root systems, correlations between spatial and fractal dimension. Am J Bot 84:26–33

Nielsen KL, Eshel A, Lynch JP (2001) The effect of phosphorus availability on the carbon economy of contrasting common bean (*Phaseolus vulgaris* L.) genotypes. J Exp Biol 52:329–339

Ostonen I, Püttsepp Ü, Biel C, Alberton O, Bakker MR, Lõhmus K, Majdi H, Metcalfe D, Olsthoorn AFM, Pronk A, Vanguelova E, Weih M, Brunner I (2007) Specific root length as an indicator of environmental change. Plant Biosystems 141:426–442

Palta JA, Berger JD, Ludwig C (2008) The growth and yield of narrow leafed lupin, myths and realities. In: Palta JA, Berger JB (eds) Lupins for Health and Wealth - Proceedings of the 12th International Lupin Conference, 14–18 September, 2008, Fremantle, Western Australia. International Lupin Association, Canterbury, pp 20–25

Probert ME, Dimes JP, Keating BA, Dalal RC, Strong WM (1998) APSIM's water and nitrogen modules and simulation of the dynamics of water and nitrogen in fallow systems. Agr Syst 56:1–28

Rengel Z, Damon PM (2008) Crops and genotypes differ in efficiency of potassium uptake and use. Physiol Plant 133:624–636

Römer W, Kang DK, Egle K, Gerke J, Keller H (2000) The acquisition of cadmium by *Lupinus albus* L., *Lupinus angustifolius* L., and *Lolium multiflorum* Lam. J Plant Nutr Soil Sc 163:623–628

Rose CW, Chichester FW, Williams JR, Ritchie JT (1982) A contribution to simplified models of field solute transport. J Environ Qual 11:146–150

Rose TJ, Rengel Z, Ma Q, Bowden JW (2009) Crop species differ in root plasticity response to localised P supply. J Plant Nutr Soil Sc 172:360–368

Sergeeva LI, Keurentjes JJB, Bentsink L, Vonk J, van der Plas LHW, Koornneef M, Vreugdenhil D (2006) Vacuolar invertase regulates elongation of Arabidopsis thaliana roots as revealed by QTL and mutant analysis. Proc Natl Acad Sc U S A 103:2994–2999

Steele KA, Virk DS, Kumar R, Prasad SC, Witcombe JR (2007) Field evaluation of upland rice lines selected for QTLs controlling root traits. Field Crop Res 101: 180–186

Tabachnik BG, Fidell LS (1996) Using multivariate statistics. Harper Collins, New York

Tang C, Robson AD (1998) Correlation between soil pH of collection site and the tolerance of wild genotypes of *Lupinus angustifolius* L. to neutral pH. Aust J Exp Agric 38:355–362

Walk TC, Jaramillo P, Lynch JP (2006) Architectural tradeoffs between adventitious and basal roots for phosphorus acquisition. Plant Soil 279:347–366

Weih M, Rönnberg-Wästljung A, Glynn C (2006) Genetic basis of phenotypic correlations among growth traits in hybrid willow (*Salix dasyclados* × *S. viminalis*) grown under two water regimes. New Phytol 170:467–477

Yuan H, Yan G, Siddique KHM, Yang H (2005) RAMP based fingerprinting and assessment of relationships among Australian narrow-leafed lupin (*Lupinus angustifolius* L.) cultivars. Aust J Agric Res 56:1339–1346

Zhu J, Zhang C, Lynch JP (2010) The utility of phenotypic plasticity of root hair length for phosphorus acquisition. Funct Plant Biol 37:313–322