Development of a novel semi-hydroponic phenotyping system for studying root architecture

Ying L. Chen^{A,D}, Vanessa M. Dunbabin^B, Art J. Diggle^C, Kadambot H. M. Siddique^D and Zed Rengel^{A,D,E}

^ASoil Science and Plant Nutrition, School of Earth and Environment (M087), University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.

^BTasmanian Institute of Agricultural Research, University of Tasmania, Private Bag 54, Hobart, Tas. 7001, Australia.

^CDepartment of Agriculture and Food, Western Australia, Locked Bag 4, Bentley, WA 6983, Australia.

^DUniversity of Western Australia Institute of Agriculture, University of Western Australia (M082), 35 Stirling Highway, Crawley, WA 6009, Australia.

^ECorresponding author. Email: zed.rengel@uwa.edu.au

Abstract. A semi-hydroponic bin system was developed to provide an efficient phenotyping platform for studying root architecture. The system was designed to accommodate a large number of plants in a small area for screening genotypes. It was constructed using inexpensive and easily obtained materials: 240 L plastic mobile bins, clear acrylic panels covered with black calico cloth and a controlled watering system. A screening experiment for root traits of 20 wild genotypes of narrow-leafed lupin (*Lupinus angustifolius* L.) evaluated the reliability and efficiency of the system. Root architecture, root elongation rate and branching patterns were monitored for 6 weeks. Significant differences in both architectural and morphological traits were observed among tested genotypes, particularly for total root length, branch number, specific root length and branch density. Results demonstrated that the bin system was efficient in screening root traits in narrow-leafed lupin, allowing for rapid measurement of two-dimensional root architecture over time with minimal disturbance to plant growth and without destructive root sampling. The system permits mapping and digital measurement of dynamic growth of taproot and lateral roots. This phenotyping platform is a desirable tool for examining root architecture of deep root systems and large sets of plants in a relatively small space.

Additional keywords: bin system, branching, *Lupinus angustifolius*, narrow-leafed lupin, root length, root morphology, root system architecture, root traits, RSA.

Introduction

Quantifying the architecture of root systems is essential because crop productivity is almost always influenced by the availability and accessibility of water and nutrients that are heterogeneously distributed in the soil (Miller *et al.* 2003; Cichy *et al.* 2009; Gregory *et al.* 2009). Root architecture directly impacts the capture of such soil resources and is, therefore, fundamental to crop productivity (Lynch 1995; Casson and Lindsey 2003; Malamy 2005; Watt *et al.* 2006; Dunbabin 2007; Fita *et al.* 2008; Gregory *et al.* 2009). Changes in root system architecture and exploitation of soil water influence the accumulation of crop biomass (Hammer *et al.* 2009).

Progress in root measurement methodology has enhanced our ability to visualise, quantify and conceptualise root architecture and its relationship to plant productivity (Lynch 1995). Numerous approaches have been developed for the study of root architecture with the support of advanced optical recording techniques. However, reliable approaches for phenotyping large numbers of plants are still being developed. Several non soil-filled methods for evaluating roots are available, including hydroponic, aeroponic and agar-plate systems (Gregory *et al.* 2009). The soil-filled root observation chambers (Manschadi *et al.* 2008) or rhizotrons (Wiese *et al.* 2005), and growth pouches (Bonser *et al.* 1996; Liao *et al.* 2004) are often used in studying roots. More recently, an improved pouch system was developed by Hund *et al.* (2009) for rapid measurement of maize (*Zea mays* L.) root systems during the first days of lateral root growth. In this system, roots grew on the surface of blotting paper facilitating the two-dimensional observation of root growth over time. However, phenotyping large sets of genotypes beyond very early growth stages remains problematic, particularly for mapping studies of quantitative trait loci (QTL).

The main objective of this work was to design and construct an inexpensive, space-saving, high-throughput phenotyping system for quantifying root architecture for large numbers of plants. This system was evaluated through a screening trial with 20 wild genotypes of narrow-leafed lupin (*Lupinus angustifolius* L.). This paper describes the construction of the bin system, along with a summary of the data that was collected in the screening experiment using this bin system.

Materials and methods

The phenotyping system

Fig. 1 shows photographs of the operational system. Each unit of the system consisted of a plastic mobile bin, support framework, 20 growth units, and a controlled watering system (Figs 2, 3). A commonly-used mobile rubbish bin (240 L, top 751×580 mm, height 1080 mm; Silverlock Packaging, Perth, WA, Australia) was chosen. The size of this bin allows for studying deep root systems (up to 1.2 m) and many genotypes within a limited space (40 plants in ~0.5 m²). Also, the bin is mobile (on wheels), which helps in moving and repositioning during the course of the experiment.

Support framework

The stainless steel supporting framework consisted of a top frame $(400 \times 600 \text{ mm})$ connected perpendicularly with two U-shaped support bars (420 mm deep) (Fig. 3). Within the top



Fig. 1. Operational semi-hydroponic bin system designed, constructed and tested for two-dimensional, non-destructive measurements of root system architecture: (*a*) a view of inside the bin system showing 10 growth units and pumping system (additional 10 units to be placed into the front part of the bin), and (*b*) side view of growth units fitted inside the bin with *Lupinus angustifolius* plants grown in the system showing the operation of the irrigation system.

rectangular frame there were 20 areas divided equally by nine parallel rods and one central dividing rod for inserting the growth units. The bottom wires of the support bars were bent in a zig-zag to hold the growth units and separate them evenly.

Growth unit

Each plant growth unit contained a 5 mm thick acrylic panel $(260 \times 480 \text{ mm})$ covered with black calico cloth $(570 \times 900 \text{ mm})$. Four 15 mm fold-back butterfly clips were used to attach the cloth to the panel on both vertical sides. The lower part of the cloth over each panel was cut in half to make two columns for root training.

Irrigation system

Each bin system was equipped with a watering system consisting of a fountain pump (Pond Max PM1000F, pump flow rate 550 Lh^{-1}) and drippers (Philmac Dripper True Drip, Philmac Pty Ltd, Adelaide, SA, Australia) connected with rigid and flexible PVC tubing. The rigid PVC tubing was secured to the middle bar of the support frame with cable ties, and the flexible tubes branched from the rigid tubing onto each dividing bar (Fig. 3). A timer was attached to the pump system for periodic water supplying.

Growth unit assembly

The growth units were positioned in the chambers against the horizontal rods and sited on the zig-zagged wire frame at the base (20 growth units per bin). The drippers located in the middle



Fig. 2. Schematic representation of the semi-hydroponic bin system: (a) Clear flat acrylic panel; (b) supporting material – calico cloth; (c) *Lupinus angustifolius* plant; (d) 240 L bin; (e) extension column of cloth; (f) water or nutrient solution; (g) pump and irrigation system. See Fig. 3 for 'support framework'.



Fig. 3. The support framework of the semi-hydroponic bin system: (a) front view, (b) side view. The rigid PVC tubing was secured to the middle bar of the support frame and the flexible tubes with drippers branched from the rigid tubing onto each dividing bar.

of the growth units were adjusted to face the growth unit. The wrapped acrylic panel with cloth formed pouches for seedlings to grow, with moisture maintained by irrigation. The growth unit allowed easy imaging down to the bottom of the plate, and the extensions of the cloth allowed room for a total length of root growth of ~1.2 m.

A list of equipment and materials needed to construct one single bin system is provided in Table 1.

System trial and evaluation

Plant materials and growth conditions

A screening experiment used two bin systems in a controlledtemperature glasshouse in June-July (20/15°C day/night temperatures) at The University of Western Australia (31°58'S, 115°49'E). Twenty wild genotypes of narrow-leafed lupin (Lupinus angustifolius L.) were randomly chosen, as representatives, from a subset of wild germplasm containing 111 genotypes to be screened for genetic intrinsic of root traits as part of an on-going research project. The 111 genotypes were previously selected using Diversity Array Technology (DArT) method which can detect DNA variability in hundreds of loci simultaneously from a large germplasm pool (1301 genotypes). Seeds were scarified by scalpel and sown in pots filled with washed river sand in a glasshouse. Seeds were germinated for 4 days and four germinated seeds of each genotype were randomly transplanted into growth units of two bins (two plants per bin). Bins were filled with 30 L solution containing (in 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 nutrient solution was supplied to the plant growth units by the automatic irrigation system through a timer controller. The periodic pumping was set as 10 min on-5 min off during the daytime (0600-1800 hours), and 5 min on-10 min off during the night time (1800-0600 hours). The solution was refreshed weekly.

Data collection

Root growth was monitored and measured weekly. The pattern of root growth on each plate was photographed by removing the cloth, and an early pattern of root growth (up to 3 weeks) was traced onto a clear panel using a marker pen. After

Fable 1.	List of equipment ar	d materials for	construction o	f inexpensive	semi-hydroponic	bin syster
----------	----------------------	-----------------	----------------	---------------	-----------------	------------

System component	Quantity	Description of part(s)
Bin	1 bin	Mobile plastic bin (240 L, top 751 × 580 mm, height 1080 mm; Silverlock Packaging, Perth, WA, Australia)
Support framework	4 pieces	Outer frame – stainless steel wire (diameter 6 mm)
	9 pieces	Horizontal rods – stainless steel wire (diameter 5 mm)
	1 piece	Central dividing rod – stainless steel wire (diameter 5 mm)
Growth unit	20 panels	Acrylic sheet — one standard sheet $(1200 \times 2400 \text{ mm})$ makes 20 panels (260 480 mm)
	10 m	Black calico cloth (1.35 m wide, Textile Traders, Perth, WA, Australia)
Irrigation system	1 pump	Water pump (Pond Max PM1000F, pump flow rate 550 L h^{-1})
	1	Timer controller
	20	Drippers (Philmac Pty Ltd, Adelaide, SA, Australia)
	5 m	PVC Drip Irrigation Tube (diameter 4 mm)
Other	22	Cable ties
	160	Foldback clips (25 mm) (Corporate Express Australia Ltd, Sydney, NSW, Australia); four per panel
	20	Foldback clips (15 mm) (Corporate Express Australia Ltd); one per panel

3 weeks, taproots of most genotypes were beyond the lower base of the plate and the length of taproot was measured. Plants were harvested 6 weeks after transplanting. The roots were subsampled by cutting into 20 cm sections along the taproot. Dry mass of shoot and roots was determined.

Image analysis

Root subsamples from harvest were scanned in greyscale at 300 dpi using a desktop scanner (Epson Expression Scan 1680, Epson America, Long Beach, Canada). Scanned images were analysed in WinRHIZO (V.2009 Pro, Regent Instruments, Montreal, QC, Canada) for root morphological and architectural studies. The debris removal filter of WinRHIZO was set to remove objects with an area smaller than 1 cm² and a length : width ratio lower than 10. Diameter classes were set to 0.17 mm (equivalent to two pixels) with 15 equal intervals. The program distinguished the taproot, lateral roots and root hairs in the images and generated root data of several traits, indicating that the image quality was appropriate. Root length data computed in WinRHIZO were compared with measurements by the Tennant's (1975) method and by manually tracing root images, with all three methods producing consistent results (data not shown).

Statistical analyses

One-way ANOVAs were performed on root traits using PASW Statistics 18 (SPSS Inc., Chicago, IL, USA). Non-significant differences were found on most of root traits between bins (95% confidence, paired-samples *t*-test) and, therefore, four plants were treated as four replicates in the analysis. The multivariate standard errors of skewness and kurtosis were 0.29 and 0.58, respectively, when all parameters were included in the analysis, indicating no significant departure from multivariate normality in the dataset. General correlations between parameters were examined with Pearson's correlation coefficients. Correlations were considered statistically significant and are indicated at *, $P \le 0.05$ or **, P < 0.01. Selected traits were subjected to principal components analysis (PCA; eigenvalue >1, Tabachnik and Fidell 1996) and *k*-means clustering analysis to compare traits with similar functions among genotypes.

Results

Root development in bin system

Growth of both shoots and roots was vigorous in the system tested. The morphology of lupin root systems was recorded photographically by removing cover cloth. Genotypic variation in root growth dynamics was apparent. The root systems of all genotypes tested primarily consisted of a taproot and first-order branches in the first 3 weeks (Fig. 4) and second-order branches were also observed for some genotypes in the later growth stage (Fig. 5*a*).

By the end of week 6, there was genotypic variation in the density of first-order branches, and in the abundance of secondorder branches. It is notable that several genotypes produced occasional fast-growing geotropic first-order branches that resembled taproots to some extent. A few genotypes also had dense root hairs usually on the first-order branches, creating an increased surface area (Fig. 5a, b).



Fig. 4. Example images showing root morphology and development of a wild *Lupinus angustifolius* genotype grown in our constructed bin system. Images were taken at (a) 2, (b) 4 and (c) 6 weeks after planting (scale bar = 5 cm).

Root trait variation

Phenotyping of 20 wild genotypes of narrow-leafed lupin produced root systems that were readily imaged and evaluated. Variations in some root traits between the tested genotypes were large (Table 2). Greatest variations were observed in the number of links, specific root length, total root length and root volume (coefficient variation, CV=0.87, 0.84, 0.79 and 0.75, respectively). Root area, link length, root tissue density, branch number and density, link area and branch intensity also differed significantly between genotypes (CV=0.67, 0.66, 0.64, 0.63, 0.58, 0.58 and 0.57, respectively). Mean root diameter showed moderate variation (CV=0.46). A large proportion of



Fig. 5. Example images of *Lupinus angustifolius* root showing (*a*) taproot, first order branches, second-order branches and root hairs on both order branches (scale bar = 5 cm), and (*b*) a close show of dense root hairs on first-order branches of a different genotype (scale bar = 1 cm).

Table 2. Descriptive statistics for root traits of 20 genotypes of narrowleafed lupin 6 weeks after planting

Branch density, the number of branches per unit taproot length; branch intensity, the number of branches per unit total root length; root tissue density, root mass per unit volume; specific root length, root length per unit mass; a link, a root segment that is between two apexes or an apex and a tip. Data of four plants for each genotype were included in analysis

	Unit	Minimum	Maximum	Mean	CV
Total root length	cm	104	2079	425	0.79
Taproot length	cm	31	111	72.6	0.26
Root elongation rate	$\mathrm{cm}\mathrm{d}^{-1}$	0.7	2.6	1.7	0.26
Root surface area	cm ²	89	1347	226	0.67
Root volume	cm ³	2.3	71.7	11.5	0.75
Specific root length	$\mathrm{cm}\mathrm{mg}^{-1}$	1.2	16	2.8	0.84
Branch number	_	120	2171	414	0.63
Branch density	cm^{-1}	0.9	9.3	4.7	0.58
Branch intensity	cm^{-1}	0.2	2.0	0.8	0.57
Root tissue density	mg cm ³	29	335	70	0.64
Diameter	mm	0.7	1.1	0.8	0.46
Link average length	cm	0.3	4.1	1.1	0.66
Link average surface area	cm ²	0.1	1.1	0.4	0.58
Link average diameter	mm	0.7	2.5	1.2	0.35
Link average branch angle	degree	33	49	40	0.07
Number of links	_	342	4485.0	721	0.87

root length occurred in diameter classes of 0.34 to 1.19 mm with 67% in relative diameter-class length (Fig. 6). A diameter class of 0.68-0.85 mm had the longest root length (20% of the total length).

Pearson's correlation coefficient analysis revealed strong correlations among morphological (e.g. root length, specific root length, root area, average diameter) and architectural traits (branch density and branch intensity) (Table 3). In particular,



Fig. 6. Mean root length in diameter class length (DCL, with s.e.) and relative diameter class length (rDCL) of 20 *Lupinus angustifolius* genotypes tested 6 weeks after planting. Data are mean across the 20 genotypes (n = 4).

the total root length was highly correlated with root area (r = 0.94), branch number (0.90), root volume (0.85) and branch density (0.84) (each at the 0.01 level of significance). Specific root length increased as numbers of branches, branch density and branch intensity increased (r = 0.77, 0.70 and 0.50, respectively, P < 0.01). Greater branch density was strongly correlated with increasing branch intensity and root diameter (r = 0.83 and 0.61, respectively, P < 0.01), and decreasing root tissue density (r = -0.61, P < 0.01).

Eleven root traits with large variations (e.g. CV > 0.50, see Table 2 for CV values) were included in the PCA analysis, which showed that 92% of the observed variation of root architecture was captured by four principal components (Tables 4, 5). The first and strongest principal component was associated with genotypes differing primarily in total root length and specific root length representing 31% of the variability. PC2 consisted

Table 3. Pearson's correlation matrix for root traits of 20 genotypes of narrow-leafed lupin 6 weeks after planting

Correlation is significant at the **, P < 0.01 level (two-tailed). Root topological traits with CV values greater than 0.45 were included in the matrix (see Table 2 for CV values). Genotype mean data (n=4) were used for Pearson's correlation analysis

	Total root length	Area	Volume	Specific root length	Branch number	Branch density	Branch intensity	Root tissue density
Area	0.94**	_	_	_	_	_	_	_
Volume	0.85**	0.97**	_	_	_	_	_	_
Specific root length	0.84**	0.80**	0.73**	_	_	_	_	_
Branch number	0.90**	0.87**	0.79**	0.77**	_	_	_	_
Branch density	0.84**	0.83**	0.77**	0.70**	0.95**	_	_	_
Branch intensity	0.55**	0.53**	0.49**	0.50**	0.81**	0.83**	_	_
Root tissue density	-0.61**	-0.51**	-0.56**	-0.58**	-0.57**	-0.61**	-0.49**	_
Diameter	0.59**	0.79**	0.87**	0.49**	0.57**	0.61**	0.42**	-0.55**

Table 4. Results of principal component analysis (PCA) on 11 selected root traits (see Table 5) of 20 genotypes of narrow-leafed lupin 6 weeks after planting – the proportion of variation explained with the first four components presented

Rotation method: varimax with Kaiser Normalization. Components with eigenvalues >1 are considered significant (Tabachnik and Fidell 1996). Genotype mean data (n = 4) were used for PCA

Component	Eigenvalue	Proportion (%)	Cumulative (%)
1	3.39	30.83	30.83
2	2.86	25.95	56.79
3	2.64	23.98	80.76
4	1.26	11.49	92.25

mostly of root diameter and volume and accounts for 26% of the variability. PC3 and PC4 add another 24% and 11% of the variability, respectively, and were strongly influenced by branch intensity (PC3) and taproot length (PC4).

Trait variation among genotypes

The tested genotypes varied significantly in the development of taproots and branches. Taproot length ranged from 31 to 111 cm with an average of 73 cm (Table 2). The total root length differed by up to 19-fold among genotypes. Clustering algorithms were used to automatically assign genotypes in terms of variability

among root traits. The *k*-means clustering analysis was performed on the global data of 10 primary traits of each genotype. The 20 genotypes tested were generally separated into two major clusters, with nine and 11 genotypes in each. Branch number, total root length and branch density were the most important contributors to this separation (F=56, 42 and 37, respectively, P < 0.001; Table 6). In contrast, taproot length and root diameter had little influence on the separation. The value of the final cluster centre indicated genotypes in cluster 1 possessed larger root systems with more root length, branch numbers, and branch density than those in cluster 2.

Discussion

The reason we developed an efficient phenotyping system was to examine genetic intrinsic variations in root traits among wild genotypes of narrow-leafed lupin and to provide basis for root models to constructing three-dimensional root architecture for selecting superior root traits for breeding program. The evaluation experiment using wild narrow-leafed lupin genotypes was to test efficiency of the established phenotyping system and the performance of wild lupin in the system. Therefore, this preliminary investigation has provided information for a follow-up large screening experiment with the same species. The rationale for the choice of narrow-leafed lupin genotypes was also because of the availability of a large germplasm pool of wild narrow-leafed lupin from landraces and diverse climatic

Table 5. Results of principal component analysis (PCA) on 11 selected root traits (see Table 5) of 20 genotypes of narrow-leafed lupin 6 weeks after planting – rotated loading scores of traits on each component

Trait/variable	Component 1	Component 2	Component 3	Component 4
Total root length	0.75 ^A	0.45	0.36	0.25
Specific root length	0.84^{A}	0.28	0.26	0.16
Numbers of links	0.64 ^A	0.45	0.47	0.29
Tissue density	-0.69^{A}	-0.25	-0.29	0.34
Diameter	0.21	0.92^{A}	0.21	-0.12
Volume	0.49	0.81 ^A	0.27	0.12
Area	0.61	0.70^{A}	0.32	0.18
Branch intensity	0.21	0.16	0.94 ^A	0.03
Branch density	0.49	0.40	0.74^{A}	0.02
Branch number	0.56	0.38	0.69 ^A	0.22
Taproot length	0.09	0.02	0.08	0.93 ^A

^AVariable loading scores with the greatest loads on each component.

Table 6. ANOVA table and final cluster centres of k-means clustering analysis on 10 root traits of 20 genotypes of narrow-leafed lupin 6 weeks after planting

Genotype mean data (n = 4) were used for k-means clustering analysis. Significant values are indicated: **, P < 0.01; *, P < 0.05

Traits	Cluster		Error		F-value	Significance	Final clus	ster centre
	Mean square	df	Mean square	df		-	1	2
Total root length	450 303	1	10673	18	42	0.00**	592	290
Taproot length	11	1	182	18	0.06	0.81	75	74
Area	130 557	1	9283	18	14	0.00**	314	151
Volume	268	1	45	18	5.9	0.03*	15	8.0
Specific root length	21	1	0.92	18	23	0.00**	4.0	1.9
Branch number	1 064 927	1	19050	18	56	0.00**	678	214
Branch density	146	1	3.9	18	37	0.00**	8.6	3.2
Branch intensity	0.68	1	0.03	18	20	0.00**	1.0	0.6
Root tissue density	22 976	1	1303	18	18	0.00**	34	102
Diameter	0.04	1	0.10	18	0.37	0.55	0.94	0.81

and geographic locations (Clements and Cowling 1991; JC Clements, pers. comm.).

Care is needed when handling growth units for observation and photography. This is because lupin roots are relatively fragile and root damage may occur. During root observations, roots and cloth remain moist, but exposure time for observation should be minimised to prevent roots from drying out. The irrigation system can be amended, e.g. by adding another opening for each unit if competition for water occurs between neighbouring plants. The volume of water or solution in the bin can be adjusted depending on the frequency of refreshment and duration of the experiment. Watering frequency and water flow can be optimised with a timer controller attached to the pump system.

Plants of wild narrow-leafed lupin genotypes were tap-rooted confirming previous observations (Clements et al. 1993). Each wild lupin genotype displayed a unique root system architecture when cultivated in our phenotyping system. The root system of each genotype retained similar morphological pattern, particularly in the proximal part, in terms of the number of the first order branches and branch angles during the 6 week growth period. We compared weekly root images and data and concluded that the 6 week root systems presented larger diversity of root traits than the early roots, although variation could be seen as early as 2 weeks after planting. Differences in root architecture among the tested genotypes primarily include taproot length, branch number and branch density. For example, some genotypes developed deeper taproot, or substantially high branch density in the upper root. Root architectural differences have significant impact on the efficient acquisition of water and nutrients in soils (e.g. Nibau et al. 2008; Rengel and Damon 2008; Rose et al. 2009), and, therefore, have relationship to plant productivity (Lynch 1995).

During the preparation of this report, we have obtained comparable data from two consequent phenotyping experiments using the same semi-hydroponic system developed in this study. The large screening experiment examined root trait variation among 111 wild genotypes of narrow-leafed lupin, followed by a repeat experiment with 10 selected genotypes undertaken in the same phenotyping system under the similar grown conditions. There was no significant difference between bins (replications) (P < 0.05). Consistent rankings of genotypes in each root trait between these two separate experiments confirmed the value of the approach for screening large numbers of genotypes using our designed semihydroponic phenotyping system. These screening experiments demonstrated a reliable and efficient phenotyping platform for the development of lupin roots. Further examinations are required to explore the potential for evaluation of root system architecture of other crop species on the phenotyping system, and to investigate the biological effects of the specific adaptations of selected genotypes with novel root traits.

Numerous rooting parameters can be collected using this binbased system coupled with a digital photography and image analysis software (e.g. WinRHIZO). The root architectural traits achieved can be divided into two general categories: topological properties (describing the pattern of root branching) and geometric properties (such as the growth angle of root axes) (Manschadi et al. 2008). Both traits are critical in studying root architecture (Lynch and Brown 2001; Kato et al. 2006; Manschadi et al. 2008). According to Fitter (1991), five types of data are required to reconstruct a three-dimensional model of a root: the number of internal and external links (without and with top meristem, respectively); the length of the links; the distribution of branches within the root, i.e. the topology; the branching angles; and the diameter per link. If one is interested in total size, rather than three-dimensional distribution, the branching angles can be left out. For the total length, rather than volume or weight, the diameters can be excluded and only the first three types of information are needed (van Noordwijk et al. 1994). Data collected from our constructed phenotyping system can be used to simulate a threedimensional model using root modelling software such as ROOTMAP (Diggle 1988; Dunbabin et al. 2002) and SimRoot (Lynch et al. 1997).

To help understand genotypic variation in root architecture and functions, routine data transformation of some traits were performed to normalise the disparity between plants of different sizes. For example, specific root length (calculated as root length divided by root mass) is an important measure of how root biomass can be used for nutrient acquisition, and is widely used in studying root architecture and functions (Ostonen *et al.* 2007). Root branch density (number of branches per unit taproot length) and branch intensity (number of branches per unit root length) govern exploration of the soil matrix and may also affect nutrient acquisition (Fitter 1991).

Unlike other hydroponic or aeroponic phenotyping systems (reviewed by Gregory *et al.* 2009), our bin system is semihydroponic and partly aeroponic. It is notable that the bin system significantly reduces environmental stresses such as hypoxia and drought effects by optimising water supply with the equipped automatic irrigation system with a controller. This system using nutrient solution has the advantage of being faster and more accurate for analysing a range of root traits, since it eliminates the challenges of soil contamination, and root loss during washing. Because the supply of water and nutrient can be adjusted for each individual bin system, there is a potential to use this system in studying root plasticity in morphological and physiological responses to water and nutrients.

To conclude, our constructed phenotyping system made from inexpensive readily-available materials was efficient in screening root traits in wild narrow-leafed lupin. It can be used to investigate relatively deep root systems and phenotype root architecture in many genotypes in a relatively small space. Furthermore, the range of root architectures observed in our phenotyping platform can be simulated using root modelling software, i.e. ROOTMAP and SimRoot, for rapid and reliable reconstruction of root systems grown in a particular environment.

Acknowledgements

This research was supported by the Australian Research Council (DP0988193). We acknowledge Dr Jon Clements from the Centre for Legumes in Mediterranean Agriculture, The University of Western Australia, and The Department of Agriculture and Food of Western Australia for providing lupin seed for this work. We are grateful to Professor Jonathan Lynch and Mr Jouke Postma of Penn State University, and Dr Jairo Palta of CSIRO for their advice and comments. Mr Ray Scott of The University of Western Australia provided assistance in constructing the bin system. We also extend our thanks to the two anonymous reviewers for their constructive and helpful comments, by which the content of this paper was much improved.

References

- Bonser AM, Lynch J, Snapp S (1996) Effect of phosphorus deficiency on growth angle of basal roots in *Phaseolus vulgaris*. *New Phytologist* 132, 281–288. doi:10.1111/j.1469-8137.1996.tb01847.x
- Casson SA, Lindsey K (2003) Genes and signaling in root development. *New Phytologist* **158**, 11–38.
- Cichy KA, Snapp SS, Blair MW (2009) Plant growth habit, root architecture traits and tolerance to low soil phosphorus in an Andean population. *Euphytica* 165, 257–268. doi:10.1007/s10681-008-9778-2
- Clements JC, Cowling WA (1991) Catalogue of the Australian lupin collection including field evaluation data for wild, semi-domesticated and fully domesticated accessions. Research Report 3/91, Department of Agriculture Western Australia.
- Clements JC, White PF, Buirchell BJ (1993) The root morphology of *Lupinus* angustifolius in relation to other *Lupinus* species. Australian Journal of Agricultural Research 44, 1367–1375. doi:10.1071/AR9931367
- Diggle AJ (1988) ROOTMAP a model in three-dimensional co-ordinates of the growth and structure of fibrous root systems. *Plant and Soil* 105, 169–178. doi:10.1007/BF02376780

- Dunbabin VM (2007) Simulating the role of rooting traits in crop-weed competition. *Field Crops Research* **104**, 44–51. doi:10.1016/j.fcr.2007. 03.014
- Dunbabin VM, Diggle AJ, Rengel Z, van Hugten R (2002) Modelling the interactions between water and nutrient uptake and root growth. *Plant and Soil* 239, 19–38. doi:10.1023/A:1014939512104
- Fita A, Pico B, Monforte AJ, Nuez F (2008) Genetics of root system architecture using near-isogenic lines of melon. *Journal of the American Society for Horticultural Science* **133**, 448–458.
- Fitter AH (1991) Characteristics and functions of root systems. In 'Plant roots: the hidden half'. (Eds Y Waisel, A Eshel, U Kafkafi) pp. 3–25. (Marcel Dekker: New York)
- Gregory PJ, Bengough AG, Grinev D, Schmidt S, Thomas WTB, Wojciechowski T, Young M (2009) Root phenomics of crops: opportunities and challenges. *Functional Plant Biology* 36, 922–929. doi:10.1071/FP09150
- 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 Science* 49, 299–312. doi:10.2135/ cropsci2008.03.0152
- Hund A, Trachsel S, Stamp P (2009) Growth of axile and lateral roots of maize. I: Development of a phenotyping platform. *Plant and Soil* 325, 335–349. doi:10.1007/s11104-009-9984-2
- Kato Y, Abe J, Kamoshita A, Yamagishi J (2006) Genotypic variation in root growth angle in rice and its association with deep root development in upland fields with different water regimes. *Plant and Soil* 287, 117–129. doi:10.1007/s11104-006-9008-4
- Liao H, Yan XL, Rubio G, Beebe SE, Blair MW, Lynch JP (2004) Genetic mapping of basal root gravitropism and phosphorus acquisition efficiency in common bean. *Functional Plant Biology* **31**, 959–970. doi:10.1071/ FP03255
- Lynch J (1995) Root architecture and plant productivity. *Plant Physiology* **109**, 7–13.
- Lynch JP, Brown KM (2001) Topsoil foraging an architectural adaptation of plants to low phosphorus availability. *Plant and Soil* 237, 225–237. doi:10.1023/A:1013324727040
- Lynch J, Nielsen K, Davis R, Jablokow A (1997) SimRoot: modeling and visualization of botanical root systems. *Plant and Soil* 188, 139–151. doi:10.1023/A:1004276724310
- Malamy JE (2005) Intrinsic and environmental response pathways that regulate root system architecture. *Plant, Cell & Environment* 28, 67–77. doi:10.1111/j.1365-3040.2005.01306.x
- 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 and Soil* 303, 115–129. doi:10.1007/s11104-007-9492-1
- Miller CR, Ochoa I, Nielsen KL, Beck D, Lynch JP (2003) Genetic variation for adventitious rooting in response to low phosphorus availability: potential utility for phosphorus acquisition from stratified soils. *Functional Plant Biology* **30**, 973–985. doi:10.1071/FP03078
- Nibau C, Gibbs DJ, Coates JC (2008) Branching out in new directions, the control of root architecture by lateral root formation. *New Phytologist* 179, 595–614. doi:10.1111/j.1469-8137.2008.02472.x
- 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. doi:10.1080/11263500701626069
- Rengel Z, Damon PM (2008) Crops and genotypes differ in efficiency of potassium uptake and use. *Physiologia Plantarum* 133, 624–636. doi:10.1111/j.1399-3054.2008.01079.x
- Rose TJ, Rengel Z, Ma Q, Bowden JW (2009) Crop species differ in root plasticity response to localised P supply. *Journal of Plant Nutrition and Soil Science* **172**, 360–368. doi:10.1002/jpln.200800031

- Tabachnik BG, Fidell LS (1996) 'Using multivariate statistics.' (Harper Collins: New York)
- Tennant D (1975) A test of a modified line interest method for estimating root length. *Journal of Ecology* **63**, 995–1001. doi:10.2307/2258617
- van Noordwijk M, Spek LY, de Willigen P (1994) Proximal root diameter as predictor of total root size for fractal branching models. I: Theory. *Plant* and Soil 164, 107–117. doi:10.1007/BF00010116
- Watt M, Kirkegaard JA, Passioura JB (2006) Rhizosphere biology and crop productivity. Australian Journal of Soil Research 44, 299–317. doi:10.1071/SR05142
- Wiese AH, Riemenschneider DE, Zalesny RS Jr (2005) An inexpensive rhizotron design for two-dimensional, horizontal root growth measurements. *Tree Planters' Notes* 51, 40–46.

Manuscript received 13 December 2010, accepted 20 March 2011