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AW Howard Memorial Trust
Grant Report

Name	Rebecca Haling
Organisation	CSIRO
Type of Grant	Grant in aid
Purpose of Grant	Prospects for improving the phosphorus efficiency of subterranean clover

Achievements

Background*:* Subterranean clover (*Trifolium subterraneum*) is grown across almost 30 million hectares of southern Australia, however, it has a high requirement for phosphorus (P) fertiliser relative to the grasses with which it is grown. Recent research has demonstrated significant variation in the ability of different pasture legume species to yield well in low P soil (Haling et al. 2016a; Sandral et al. 2018; 2019). Species (e.g. *Ornithopus* spp.; serradellas) that can efficiently maximise soil exploration by combining high root length density (i.e. root proliferation) with high specific root length (i.e. more root length per unit root dry mass) and long root hairs can capture more P and yield well in low-P soils. Subterranean clover genotypes that combine high root length density and high specific root length yield relatively well in low-P soil. However, the root hair length of subterranean clover is very short; (0.2-0.4 mm) compared with more P-efficient legumes (e.g. serradellas, 0.6-0.8 mm) and prevents them from achieving high P-acquisition efficiency (Haling et al. 2016b; 2018). This grant was used to search for subterranean clovers in the Australian Pastures Genebank that have root traits adapted to low soil P fertility. The work was particularly focused on attempting to identify accessions of subterranean clover with long root hairs.

Methods and Results*:* Recent research in a national P-efficient pastures project[1,2] screened a 'core collection' of subterranean clover (97 accessions; ~80% of the species diversity) for root acclimation and improved yield in low-P soil. As part of this AW Howard grant, a preliminary analysis was undertaken to identify whether there was an association between the collection location of the 95 wild accessions in this 'core collection' and the root traits and/ or yield of these accessions in low-P soil. The hypothesis was that if characteristics of plant collection locations were correlated with low-P root acclimation traits, they may be useful predictors of genotypes adapted to low-P soils. Data were analysed to investigate whether there was an association between known (i.e. information collected at time of collection of the accession) and/ or predicted (i.e. modelled) bioclimatic and soil parameters at the sites, and root traits and/ or yield. However, no consistently strong link was found. The lack of strong association may, in part, be due to (i) the low accuracy of the environmental parameters collected with lines in the Genebank, (ii) low accuracy of environmental parameters predicted (i.e. modelled, not measured) for collection locations, and/or (iii) lack of relevance of these parameters for predicting P efficiency traits.

Consequently, a second approach was developed based on selecting accessions of subterranean clover that were collected at sites where other P-efficient pasture legumes had been collected. Passport information for all accessions of subterranean clover held in the Australian Pastures Genebank was obtained. The list of accessions was initially limited to those collected overseas (i.e. wild accessions; approximately 7000 accessions collected mainly around the Mediterranean basin) and then further limited to those that were listed as being collected at locations where *Ornithopus* spp. (*O. compressus*, *O. pinnatus*, *O. perpusillus*, *O. sativus*) were noted as growing (~960 accessions). Most of these sites were recorded as being 'sandy', which may also indicate nutrient-poor soils, and the sites were often noted as having other P-efficient legume species growing at them; e.g. *Lupinus* species.

For practical reasons, the number of accessions was limited to include only a single accession from each location and only subterranean clovers of the subspecies *subterraneum* (~320 accessions). Seed was obtained for 260 of these accessions. The remaining lines of interest were unavailable due to low seed quantities.

The 260 accessions of subterranean clover were subsequently screened for root hair length. Briefly, approximately 20-30 plants per accession were established in a pot (20 cm height; 8 cm diameter) of a sandy loam soil that had been amended with lime (to raise pH to 5.5; $CaCl_2$-extractable), phosphorus (to achieve an "intermediate" level of soil available, Colwell-extractable P of ~24 mg P/kg) and other basal micro- and macro-nutrients. Controls with known "short" (*T. subterraneum* cvs. Losa, Leura, Bindoon, Riverina, Mt Barker, Dalkeith), "intermediate" (*T. meduseum* and *T. pauciflorum*) and "long" root hairs (*O. sativus* cv. Margurita and *O*. *compressus* cv. Santorini) were also grown. Plants were inoculated with rhizobium. Plants were grown for 2 weeks in a controlled environment cabinet (15-20°C night/ day temperature 12/12 hrs dark/light). At harvest, soil was removed from the pots as an intact core and the soil core was cut approximately a third of the way from the top of the pot. Roots were gently washed from the bottom two-thirds of the soil core and stored in water at 4°C prior to assessing root hairs. The plants were returned to pots with additional soil and were maintained in the growth cabinet until root hair assessments were completed. The length of root hairs was ranked under a dissecting microscope using a graduated eyepiece with 0.25 mm increments. Root hairs were assessed on the taproot (lateral roots had only just started to elongate) at a distance from the root tip where root hairs were fully elongated. Root hairs on each accession were classed as 0.25, 0.375, 0.5, 0.75 or 1 mm in length to facilitate rapid assessment of root hair length on a large number of plants.

The root hair lengths of the control genotypes were consistent with their previously determined classification of "short" (0.2-0.4 mm), "intermediate" (0.5-0.6 mm) or "long" (>0.6 mm) root hair genotypes (Table 1). Among the accessions of subterranean clover, the vast majority (249 of the 259 that grew) were ranked as having "short" root hairs. Nine accessions had root hairs that were "intermediate" in length (0.5 mm) and a single accession was observed to have several plants with "long" root hairs (Table 2). The plants of this accession have been retained, and additional seed of this accession has been requested from the Australian Pastures Genebank. Further studies will be undertaken to establish whether these plants are true, 'outliers' in terms of root hair length, or if an artefact of the pot environment has resulted in these apparently long root hairs (e.g. root hairs growing into a pore space).

Table 1 Root hair length scores of control genotypes.

Control type	Genotype	Root hair length score (mm)
Short root hairs	*T. subterraneum* cv. Leura	0.25
	cv. Bindoon	0.25
	cv. Riverina	0.25
	cv. Mt Barker	0.29
	cv. Dalkeith	0.33
	cv. Losa	0.38
Intermediate	*T. pauciflorum*	0.50
	T. meduseum	0.50
Long root hairs	*O. sativus* cv. Margurita	0.88
	O. compressus cv. Santorini	0.92

NB: Results for the control genotypes is based n= 3 replicate pots. A single pot was established per accession reported in Table 2.

Table 2 Frequency of root hair length scores for subterranean clover accessions.

Frequency	Root hair length score (mm)
162 accessions	0.250
87 accessions	0.375
9 accessions*	0.500
1 accession	0.750

* For 3 of these accessions, root hair lengths of 0.5 mm were only observed on 2 of the ~20-30 plants in the pot. NB: Seed of one of the 260 accessions was not viable.

Outcomes

The grant was used to target and then screen 260 wild accessions of subterranean clover from the Australian Pastures Genebank for long root hairs. Developing subterranean clovers with long root hairs is considered key to achieving a step-change in P efficiency of this important pasture species. The work has tripled the total number of accessions of subterranean clover that have now been screened for root hair length.

Results have confirmed those of earlier studies that found that the range in root hair lengths among subterranean clovers is likely to be limited. The vast majority (96%) of accessions screened in this work were found to have root hair lengths in the previously characterised range of 0.2-0.4 mm. Nine accessions had root hair lengths ~0.5 mm, however, these lengths are unlikely to be sufficiently long to achieve a step-change in P efficiency in subterranean clover. A single accession was observed to have root hairs ~0.75 mm in length along some sections of roots. A true "outlier" genotype is required to match the root hair lengths found on serradellas which are highly P efficient. Further studies will now be undertaken to establish whether this is a stable outlier with long root hairs, or whether the observation was transient (either not a stable trait or an artefact of the plant growth conditions).

The grant was also used to attempt to develop other targeted approaches to identifying accessions in the pastures genebank with root traits of interest. Preliminary analysis indicated that for traits relating to P-acquisition efficiency, the relevance of available environmental parameters and/or their accuracy is inadequate to develop such an approach. However, while this component of the work was not successful, the work highlighted potential approaches to achieve greater and potentially more efficient utilisation of plant genebank resources. The work also highlighted the general importance and value in maintaining resources like the Australian Pastures Genebank for novel trait discovery.

The results of this work indicate that, except for one accession that is currently undergoing further studies, there is limited range in root hair lengths among most subterranean clovers. If the root hair phenotype of this accession is found to be stable, studies will be undertaken to assess whether the longer root hairs confer greater P acquisition efficiency, and the prospects for using this accession to breed for more P efficient subterranean clovers. However, if this is not found to be the case, this will be a valuable outcome as it would confirm that introducing and developing serradellas as an alternative legume for the permanent pasture systems of southern Australia is currently the only viable option to reduce P fertiliser costs in these systems. Some serradellas can yield as well as subterranean clover with approximately a third less P fertiliser inputs. With P fertilisers currently representing approximately a quarter of the variable input costs in Australian pasture systems and with significant pasture yield gaps attributed to nitrogen deficiency, developing viable alternative legumes to augment the use of subterranean clover is important for continuing productivity, profitability and resilience of the grazing industries in southern Australia.

Outputs: None to date. Future work is planned with the accession observed to have long root hairs.

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[1] In collaboration with researchers at the University of Western Australia and NSW Department of Primary Industries, and with funding support from the Australian Government Department of Agriculture as part of its Rural R&D for Profit programme and from Meat and Livestock Australia, Dairy Australia, and Australian Wool Innovation Limited as part of the project "RnD4P-15-02-016 Phosphorus Efficient Pastures" (2016-2020).

[2] In collaboration with researchers at the University of Western Australia and NSW Department of Primary Industries, and with funding support from Meat and Livestock Australia, and Australian Wool Innovation Limited as part of project B.PUE.0104 (2013-2017).

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